




Validation-data Generation for Brightfield Microscopy Cell Tracking using Fluorescence Samples

Generación de Datos de Validación para Rastreo Celular en Microscopía de Campo Claro usando Muestras Fluorescentes

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Keywords

Brightfield microscopy; cancer; fluorescence microscopy; pattern recognition.

Abstract

This work focuses on the use of fluorescent cancer cell images as data to validate the results obtained in segmenting brightfield cancer cell images, as the latter's current validation consists of manual annotation of cells in the original images. The procedure uses pattern recognition and starts with preprocessing the fluorescent samples to ensure cell detection, focused on area and intensity value. As the fluorescent images are segmented, each cell's nucleus is detected and counted, with a high success rate as each nucleus's contour was detected with its original shape. As each image's density is calculated, they can be clustered according to their density value and used for cell detection in brightfield samples.

Palabras clave

Cáncer; microscopía de campo claro; microscopía de fluorescencia; reconocimiento de patrones.

Resumen

Este trabajo usa imágenes de fluorescencia de células cancerígenas como para validación de resultados obtenidos por segmentación de imágenes de campo claro de células cancerígenas, ya que actualmente la validación consiste en la anotación manual de las imágenes originales. Se usó reconocimiento de patrones y se inició con preprocesamiento de muestras fluorescentes para asegurar la detección de células, considerando área y valores de intensidad. Al segmentar las imágenes de fluorescencia el núcleo de cada célula es detectado, con su forma original. Al calcular la densidad de cada imagen, estas pueden ser agrupadas de acuerdo a su valor de densidad y usadas para la detección de células en muestras de campo claro.

Introduction

Cancer occurs when damaged cells are able to bypass checkpoints and replicate out of control, transmitting the damage. When cancer is considered as a possible diagnosis, part of the affected tissue is analyzed and maintaining the genetic material's integrity is essential.

The analysis of cell samples is a complex process that depends on the technique used to see them. The genetic material must retain its integrity to obtain valid results, so an appropriate technique to visualize the samples is brightfield microscopy. This technique is used as it is of low cost and the sample isn't damaged. The brightfield images studied for cancer make analysis difficult, they present low contrast and a density that increases through time, as a study can last about 92 hours. These images also have noise and illumination irregularities, the cell's contours can't be properly defined and the nuclei are not distinguishable.

Fluorescence microscopy is a technique that in these cancer samples shows nuclei, with different levels of absorption for each cell. This technique, although it allows the visibility of the cells to be studied, compromises their DNA integrity.

Considering that throughout the study, images in brightfield and fluorescence microscopy are obtained, the purpose of this project is to use microscopy segmented images as validation data to corroborate the results in bright field segmented images, and guarantee a cell tracking algorithm's validity. It also speeds up the process of obtaining validation data, as the current way involves hours of manually annotating cells in brightfield microscopy images.

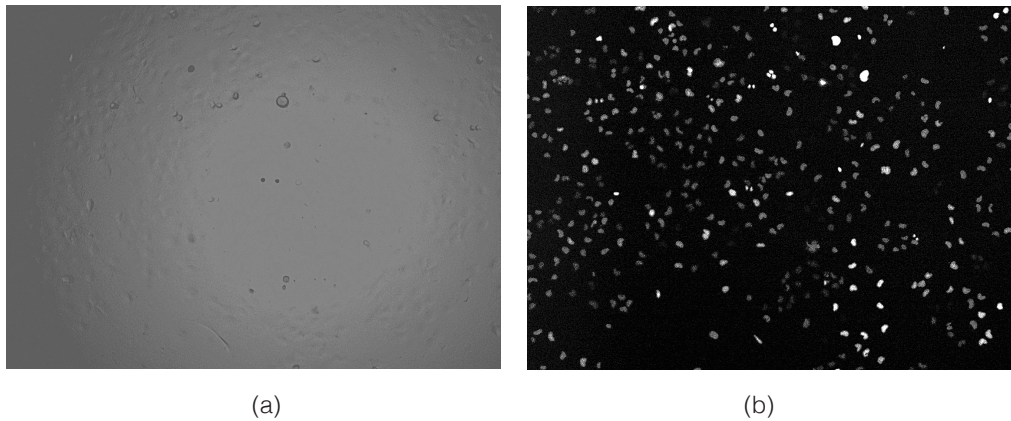


Figure 1. Samples obtained through (a) Brightfield microscopy and (b) Fluorescence microscopy

Related Work

Commercial solutions for analysis of cell samples do exist and most of them focus on using additives as staining or wave producing filters to obtain fluorescent samples [1]. The problem is that this method cannot guarantee or protect the integrity of the genetic material in the cells, so a complete analysis is difficult to perform.

According to Zuiderveld [2] and Kim [3], CLAHE Equalization divides the image in blocks and performs the histogram equalization, avoiding noise amplification. Some studies that used fluorescence microscopy show techniques as anisotropic smoothing, gradient mapping and the use of characteristics as size and shape [4], [5]. Other solutions [6] could be used for noise removal and edge preservation. The deceived bilateral filter allows noise reduction and contrast increase showing the benefit of a good preprocessing phase can overall help the tracking algorithm in its search of the image's cells [7].

For unstained cell detection as segmentation is needed, Espinoza worked on an alternative that used of a local threshold after the global threshold is estimated considering contours [8]. Operations in this and in [9] come with a computational price, as they are applied first to the whole image and then to different regions in the image separately and sequentially. Contour definition and center detection were considered ideal to segment cells separately from the cluster in high density images [10], [11].

Methods

For cell identification in fluorescent cell images, it is necessary to first implement a preprocessing phase, to guarantee the segmentation and detection of the cells in the images. The next steps are followed to ensure cell detection:

1. The bilateral filter for smoothing and erasing noise while preserving contours. The Canny filter with a Gaussian filter helps define the nuclei's contours in preparation for segmenting.
2. The segmentation using the Otsu technique can obtain a binary image, resulting in white objects and black background.

Detection of cells in brightfield microscopy and their nuclei in fluorescence microscopy considers their intensity and size. As the objects are detected, their positions in the images are preserved for future procedures. Through the analysis of different images throughout the study, we can classify the images according to their density, based on the number of nuclei detected.

$$density = \frac{cellpixels}{totalpixel}$$

Equation 1. Image density.

As this value is obtained for each image, they can be classified and obtain the segmentation's result according to how many cells each image has, and test the validity of the method depending on the image's density. The validation of the methodology followed must detect any error present in the procedure, as they translate into its utility. For a quantitative validation manually annotated cells fluorescent images are used to compare the number of cells found and their position in the image.

Experiments and Preliminary Results

To obtain the cells present in a Brightfield cell samples, the algorithm for pattern recognition is followed, with intensity, shape and size as special characteristics considered (Quinde-Cobos, 2020). These samples are characterized by their low contrast and increase in density as the study advances. Because of this, the complexity of the algorithm increases, which has to be covered in the detection and tracking algorithm.

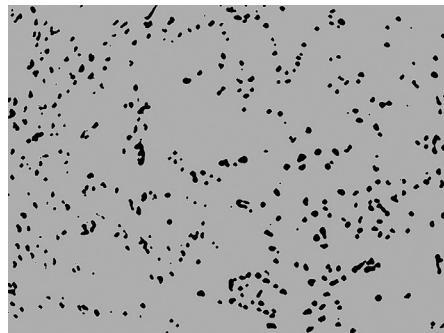


Figure 2. Objects detected for Brightfield image

Figure 2 shows a preliminary result of Brightfield sample's segmentation of high density. In comparison to the manually annotated image, a significant percentage of the cells were not detected, which showed the necessity of the fluorescent sample's segmentation as validation data to make easier the analysis of results as shown in the image.

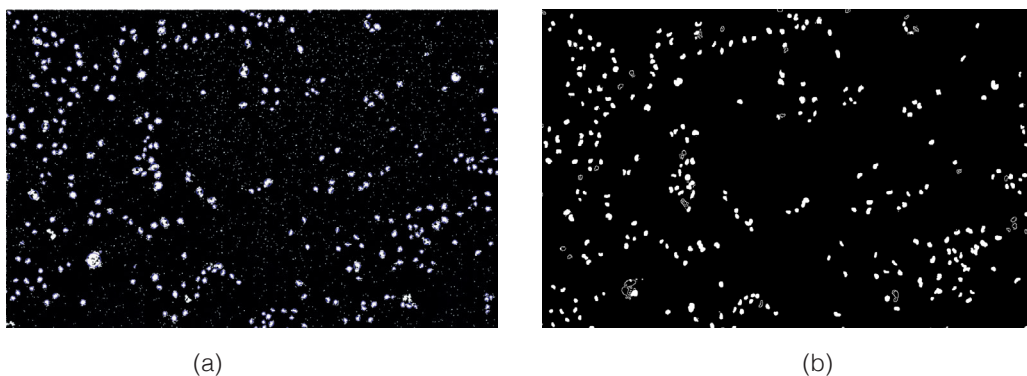


Figure 3. Objects detected for fluorescent images

The figures 3.a and 3.b show objects detected for an image obtained through fluorescence microscopy. In 3.a, each detected cell has a blue circle over it to show that it was properly detected. Morphology operations were applied which modified the contours' original shape. In 2.b, each cell was identified because of their contour, which allows to maintain their original shape but limits their identification to the detection of the contours. In comparison to their annotated images, the second method provided a better detection for the objects present, which represent each cell's nucleus.

Conclusions and Future Work

The basic structure of the pattern recognition process was used to create each segmentation's algorithm. These algorithms, created for these specific cancer cell samples, try to eliminate noise or other hindrances present in the images to facilitate the object detection process. For each image, the present cells were obtained and their position in the image saved for validation and future procedures.

With the detection of the objects present in the fluorescent images, an analysis of the density ratio according to the frame number must be performed, to detect clustering in density values and divide them into groups, resulting in a classification of the images based on their density, which can help create a personalized method according to this measurement. These classification can be applied to an algorithm for brightfield cell detection and tracking to add robustness and validate the results and objects found in each sample.

Measurements of density may vary depending on the type of cancer studied and how fast cells replicate according to the type analyzed. Because of that, to each study analyzed the density values must be obtained to ensure the images classification according to the number of objects present.

The data managed in this work differ from the data analyzed in previous studies, given the complexity of their features and variations along the samples examined. Even though the method followed may appear outdated, according to the literature consulted it gives the desired result.

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