Development of An Image Analysis Methodology For Animal Cell Cultures Characterization

P-BE6

A.L. Amaral[1,2], D.P. Mesquita[1], M. Xavier[1], L. Rodrigues[1], L. Kluskens[1], E.C. Ferreira[1] (1) CEB - Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal, (2) Instituto Politécnico de Coimbra, ISEC, DEQB, Rua Pedro Nunes, Quinta da Nora, 3030-199 Coimbra, Portugal; lpamaral@*isec.pt.

To establish a strong cell culture protocol and to evaluate experimental results, a quantitative determination of animal cells characteristics, such as confluence and morphology is quite often required. Quantitative image analysis using automated processing has become a routine methodology in a wide range of applications with the advantage of being non-invasive and non-destructive. However, in animal cells cultures automatic techniques giving valuable information based on visual inspection are still lacking. In the present work an image analysis procedure was developed to accurately detect animal cell cultures from images captured in phase-contrast microscopy.
Image analysis results demonstrated that the methodology was successfully applied, leading to more efficient animal cell culturing with less variability.

Background

In the specific case of animal cell cultures, the development of robust procedures relies on the ability to accurately assess characteristics of adherent cell populations. This information is required both to ensure consistency during routine maintenance, and to assess the outcome of experimental investigations. Classical assays, such as cell enumeration using a counting chamber, require detachment of the cells and are thus disruptive to key characteristics of the cell populations, such as distribution or morphology, preventing the collection of this potentially valuable information [1]. Current research on monitoring of animal cell cultures benefits from a non-invasive and non-destructive analytical method for rapid and precise determination. The most common way to assess properties of adherent cultures is the inspection by light microscopy. Through these inspections, it is possible to estimate the confluence, a measure of the fraction of the growth area covered by cells. Thus, confluence is particularly useful when detachment is not possible and for example to determine when to transfer the cells to other flasks [2]. Cells morphology is also an important characteristic mainly to distinguish different type of adherent animal cell cultures.

Nowadays, computers are key equipments for the analysis of tasks requiring complex computation, and for the extraction of quantitative information, opposite to the qualitative evaluation of human analysis. The automatic analysis of images captured by digital cameras enables us to quickly extract quantitative information [3]. Hence, as a basic concept, image processing and analysis is the extraction of significant information from images, by means of digital image processing techniques. The diversity of digital image processing and analysis applications is continuously growing through all areas of science. Regarding the use of this technology, it is essential to take into account the early stages of image acquisition and image processing. These steps are essential to obtain good quality images and extract the most important information.

Objectives

Based on the recent advances in quantitative image analysis (QIA), we developed a new algorithm to accurately assess confluence and to study the morphological characteristics of different animal cell cultures. Size related variables, and morphology-related parameters indicating the space fulfilling ability and the elongation of the cells were also evaluated alongside confluence determination.

Methods

Different cells were used – MDA-MB-231 and -435, both cancer cell lines, and MCF-10-2A, a non-tumorigenic line. Cells were observed in a Leica DM IL inverted microscope, in phase-contrast at 100x total magnification, coupled with a Leica D-LUX 3 camera, ensuring equal acquisition conditions. The image processing and analysis programs were developed in Matlab 7.8.0 (The Mathworks, Natick, MA), and based on the

cells identification, quantification and morphological characterization.

Phase-contrast image processing

A detailed description of the developed image processing and analysis program is presented below (Figure 1).

Pre-treatment – The image pre-processing stage depends on the enhancement of grayscale images to improve the boundaries contrast. For that purpose a bottom-hat filter was used, so that boundaries between cells are highlighted.

Segmentation – A combination of different functions was used for cells edge determination. Upon the segmentation of the bottom-hat image, a combination of a median filter, hole-fill and reconstruction procedures was then performed to determine the cells region of interest. This latter image was used as a mask in the bottom-hat filter to further allow for a second, more precise, segmentation procedure. Furthermore, the application of hole-fill procedure, erosion and reconstruction morphological operations was further used, to remove small debris. The resulting binary image was then used for confluence determination. Where morphological characterization of cell cultures was required, a step to suppress the objects connected to the image border was applied prior to the final binary image.

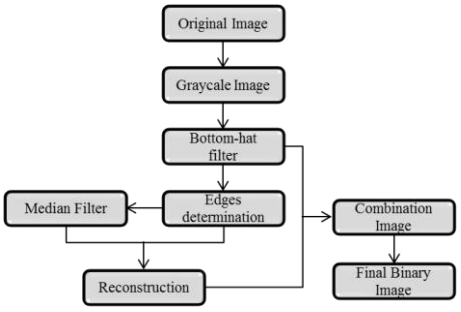

Figure 1. Schematic representation of the image analysis procedure.

Morphological Characterization

Several parameters to characterize the cell cultures were evaluated following a previous work of Amaral (2003) [4]. The main parameters determined were the cell area, equivalent diameter, perimeter, length and width (size related variables), solidity, roundness, compactness (space fulfilling ability), extent, eccentricity (elongation of the object), convexity (roughness of the object's borders), and robustness (conjugates the fulfilling ability and the border's roughness).

Results

One of the major concerns using QIA is the number of images necessary to characterize and provide representative information about cell populations. Since the culture flasks may have different sizes, a prior study on the number of images randomly selected from the same culture flask was performed (Figure 2). As can be observed, the confluence percentage of the cell culture was around 23% for the selected images. Keeping in mind that lower image numbers increase the associated error, it could be established that for a minimum of 16 images an acceptable 5% error was obtained indicating the feasibility of the present image analysis methodology even with a low number of images.

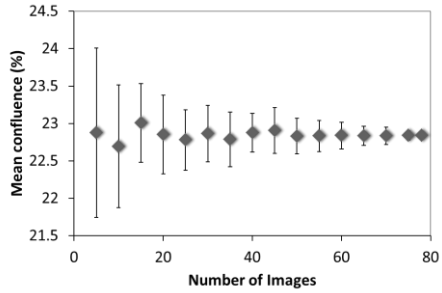

Figure 2. Confluence percentages obtained as a function of the number of images.

Regarding the morphological characterization of animal cell cultures, it was proposed to detect the morphological changes depending on the type of cell under research. This topic is now in progress with successful preliminary results.

Conclusions

With the present QIA methodology, a non-invasive and non-destructive process was developed to monitor animal cell cultures. Margin errors (5%) on the mean confluence determination were obtained with the reduction to a total of 16 images for the same flask of cell cultures. The assessment of morphological characteristics is still in progress, and the preliminary results show the main differences on the three animal cell cultures.

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