Morphological Plant Cell Analysis allowing a better control of the bio-process *

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Résumé

En biologie, il est important de disposer de mesures statistiques pour contrôler un processus biologique. Les mesures manuelles sont pénibles, imprécises ou même impossibles. Dans cet article, un système automatique pour l'analyse de cultures de cellules est présenté. Les images sont rehaussées en utilisant la transformation morphologique Chapeau Haut de Forme et la transformation de Hough. Le traitement est robuste et efficace et ne détruit, ni ne change ou enlève des cellules. La segmentation est obtenue en utilisant l'algorithme morphologique de partage des eaux. Vu leur grande variété, il est préférable de séparer les grandes et petites cellules pour les segmenter. Pour chaque classe de cellules, un algorithme pour l'extraction de marqueurs est élaboré.

Abstract

In biology, it is important to have statistical measures to control a bio-process. Performing these measures by hand is a fastidious, imprecise or even an impossible task. In this paper an automatic system to analyse a given cell culture is presented. The images are enhanced using the morphologic Top-Hat transformation and an adaptive form of the Hough transformation. The processing proves to be efficient and does not destroy, alter or remove any cells. Segmentation is obtained by using the morphologic watershed algorithm. Due to the large variety of cells, it is preferable to separate and segment small and large cells individually. For each class of cell a specific marker extraction algorithm is elaborated.

1 Introduction

There is a pletore of application for image analysis in order to classify differently shaped and sized objects, such as in medical image analysis, quantitative cytology, metallurgy, biology and others. In the work described here we are situated in the biological environment.

Biologists are currently working on the cultivation of naturally pigmented cells with a possible application in the food industry. The cells are cultivated in so called bio-reactors which contain billions of cells, making a manual analysis impossible. Image analysis will allow a finer control of the bio-process. To date, only little has been done in this field. In [1], skeleton length measurements were made after segmentation by thresholding.

The goal of the analysis presented here is to classify a culture scene according to shape and grey level features and to retrieve relevant statistical data on each class, such as length, width, number, area of cells. The analysis is performed in five steps:

- Image Enhancement
- Segmentation
- Feature extraction
- Fuzzy Classification
- Statistical measures

This paper is concerned only with the first two points. Enhancement is presented in section 2. The segmentation algorithm is introduced in section 3 and the marker extraction algorithms are presented in section 4. Finally, conclusions are drawn in section 5.

2 Image Enhancement

2.1 General Considerations

An imaged cell culture sample is shown in Figure 1 top left, corresponding to a real scene diameter of 1.5 cm. Unfortunately, the image is of rather poor quality and due to the thick liquid sample, improved illumination conditions are difficult to obtain. Thus, it will not be possible to isolate cells from the background by thresholding, as explained, for example, in [2, pp.61–73] and [3][4].

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In this section, a possible method based on the morphological Top-Hat Transformation is presented to eliminate the non uniform background. The detection of the useful scene is performed using the Hough Transformation.

2.2 Background enhancement: The Top-Hat transformation

This morphological transformation has been presented for the first time by Meyer in [5]. Sometimes it is also called the rolling ball transformation [6]. It is defined as

$$h = f - \gamma_B(f) \quad (1)$$

where f is the initial function and $\gamma_B(f)$ is the opening of f. The function $\gamma_B(f)$ is defined as the erosion of the original image followed by a dilation: $\gamma_B(f) = \delta[\epsilon(f)]$, where $\delta(f)$ and $\epsilon(f)$ denote the morphological dilation and erosion, respectively. The only parameter in this system is the size of the structuring element for the opening. Experimental results have shown that there are always excellent enhancement results obtained even when taking large security factors.

2.3 Extraction of the useful scene: Hough Transformation

The Hough Transformation[7][8] is a very efficient algorithm to extract curves of known parametric representation from an image. Curves like lines, circles, parabola, etc. can be detected. An adapted implementation using a small parameter space allows a very fast convergence. The final enhanced image is shown in Figure 1.

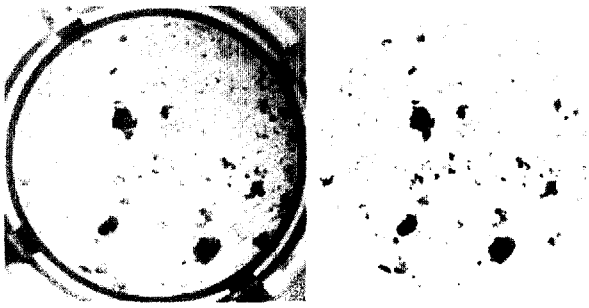

Figure 1: Original and enhanced image

3 The Watershed Algorithm

The morphological segmentation algorithm shows parallels with how man segments an image. This may be described as a coarse to fine procedure. By only taking a glimpse at an image, we are not able to delimit every object exactly, but we are able to give an approximate indication of its position. Later, by taking more time to look at the image, we are able to locate frontiers due to significant changes in luminance. Translating this knowledge into mathematical terms leads to the watershed algorithm[9]. The main characteristics are summarized in the next paragraph.

In grey level images, frontiers correspond to significant changes in grey between two adjacent regions. These are detected by the gradient image. If seen as a topological surface, then each object of the original image becomes in the gradient image a regional minimum, called a catchment basin, surrounded by a chain of mountains. It seems natural to consider the crest line of this mountain chain as the boundary. It is called the divide line. Unfortunately, a problem subsists when applying this idea to real images. The non-uniform grey level of a cell implies several regional minima. The watershed algorithm will detect all of them which results in a severe oversegmentation. Therefore, a smaller number of valid catchment basins must be selected, by eliminating undesirable minima in the gradient image that do not correpspond to a cell.

The algorithmic implementation does not require one minimum per cell but one connected area per cell. We will call this area a *marker* of the cell. Let us view it as the intelligent part. In a second step, the watershed algorithm uses the marker and the gradient information to segment the image. This is the mechanical part.

In the next section we attempt to find a procedure for the intelligent part, i.e. to isolate one marker for each cell.

4 Marker extraction

4.1 General considerations

Several procedures can be thought of for immediate marker detection. A preliminary filtering of the original image by a median filter or an opening, for example, reduces the noise in the image. The choice of general parameter values, however, for such a method is difficult or impossible and results are far from being satisfactory.

In the present case, a better basis for marker extraction can be established by analyzing large and small cells separately, that is cellular aggregates and individual cells, respectively. Single cells may be classified as rods or spheres.

4.2 Separation into aggregates and rods

When looking at the plant cell images, we remark that there are three different cell constitutions:

1. Single standing rods
2. Rods superposed on aggregates
3. Aggregates

They will be processed and extracted in this order.

Single standing rods are detected using the next equation:

$$g = f - R_{max,f}(R_{max,f}(\gamma_n(f))) \quad (2)$$

where $\gamma_n(f)$ is an opening of f with size n, and $R_{max,f}(g)$ is the maximal geodesic reconstruction of g with regard to the mask f.

The maximal geodesic reconstruction differs somewhat from the original reconstruction [10]. Before the reconstruction takes place, all the points of the marker function are put at the maximal grey value. The reconstruction iteratively dilates the marker function with the restriction to be inferior or equal to the original function. The maximal reconstruction will thus exactly represent the original function over the initial valid marker domain as shown in Figure 2.

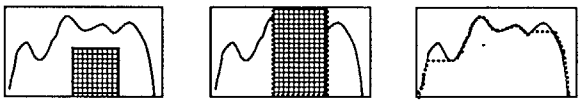

Figure 2: Maximal reconstruction of a marker

In a second step we will extract the superposed cells. In addition to shape, another criteria is introduced relating to the difference in grey value. First, all possible candidates are selected that fulfill the shape criteria. Secondly, among these cells only those having a significantly different grey value compared to their neighborhood are retained. A modified Top Hat transformation is used which is less sensitive to undesirable artifacts. The modification is based on the following observation: Between two superposed rods, there is always a distance of at least the size of a rod between them. Hence, any two peaks closer to each other than this critical distance can be discarded.

This can be achieved by preceding the opening by a closing. In order to obtain an anti-extensive transformation, we take the minimum between this result and the original function. This modified Top-Hat transformation can be written as follows:

$$g = f - (\gamma\varphi(f) \wedge f) \quad (3)$$

where $\wedge$ denotes the inferior value.

In order to take into account the fact that superposed cells have a significantly different grey level than their neighborhood, a simple thresholding of g yields very good results.

$$h = threshold_H(g) \quad (4)$$

The threshold may be set at a rather high value assuring that contrasted objects are detected exclusively. A high threshold can generally be used over a wide range of images and is not critical at all. The resulting image contains small cells only. However, due to the high thresholding, it is very likely that part of a cell will be cut off. In order to reconstruct the true shape of all remaining cells, the geodesic reconstruction is the tool to be used.

$$s = R_g(h) \quad (5)$$

The union of the two images with small single standing cells gives all small cells, as shown in Figure 3.

Large cells are obtained by simply taking the difference between the original image and the image with the small cells. Large cells which had small cells superposed will now contain a hole. These holes may be filled by a conditional closing operation.

$$l = \varphi(f) \wedge f \quad (6)$$

The resulting image with only large cells is presented in Figure 3. Eventually, we have two images that are suitable for segmentation.

Figure 3: Separated Aggregates and Rods

4.3 Marker for aggregates

Analysis of the images characterizes large touching cells by the following two conditions:

1. Even in the smoothed image they have significantly different grey values.
2. An erosion of a certain size separates them

We will exploit these two properties in order to find the remaining aggregates.

Let us start with the erosion operation which separated two connecting large cells.

$$e = \epsilon^N(f) \quad (7)$$

where N corresponds to the size of the erosion. Unfortunately, there are also large cells divided into two that actually are only one cell. These are the ones that have a form as illustrated in Figure 4.

These particules should be merged together using the grey level information. If the erosion operation leads to splitting a cell into two, but the two parts are of similar grey value, then the splitting should be canceled. In other words, if the gradient in the original image is

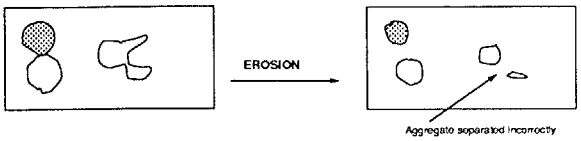

Figure 4: One large cell that is divided in two by erosion

below a certain threshold, then the eroded image has to be restored with the original value. This operation of checking the value of the gradient should be performed everywhere that the erosion has taken away part of the particle. If the erosion has taken place with a structuring element of size N, then the gradient must be at least of size N. This can be obtained by the difference between the original and an erosion of size N.

$$g = f - \varepsilon^N(f) \tag{8}$$

The eroded image will be restored at the original value, if the following holds:

$$e = f \qquad if \quad g < threshold \tag{9}$$

Hence, all the connected particules are markers for the watershed algorithm. The gradient image for the watershed is the two pixel width morphological gradient of the original image.

The segmented image is shown in Figure 5.

4.4 Marker for small cells

The main problem is the restricted size of the objects. A rod may be as small as three to four pixels in width, and hence morphological operations with small structuring elements can already make them disappear. Furthermore, the cells are not convex, nor do they present a regular geometric form. The algorithm, as previously elaborated for the aggregates, does not work because there are cells touching that have practically the same grey level and hence several cells would be merged.

In order to obtain markers we exploit a particular property of the small cells that was observed over a large range of different cell cultures. If seen as a topographical surface, each cell represents a little mountain chain. Every mountain has a decreasing width as we approach higher altitude. On the other hand, the crest line between two adjacent cells is much larger. By applying a Top-Hat transformation of size one, the crest line of every cell is extracted and two different cells result in two different connected markers. The final segmentation result is shown in Figure 5.

5 Conclusions

This work allows a practical application of Mathematical Morphology and shows its exciting possibilities.

Figure 5: Segmented Aggregates and Rods

A method of enhancing poorly illuminated images is explained. It is carried out using the Morphological Top Hat transformation. Segmentation is performed separately on the small cells and the larger cellular aggregates using the watershed algorithm. Two different marker extraction algorithms take into account the spatial properties of each one of these cell categories.

In practical application, it is particularly important to have only little system parameters to adjust. This requirement is well fulfilled in this work.

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