Automatic classification of endothelial and fibroblast cell culture images

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Abstract. In the paper we searched for the subset of features suitable for the automated classification of endothelial and fibroblast cell culture images. The overall image classification is composed of the two main steps. First, each of the cell culture is classified by its confluence character. Secondly, confluent endothelial cell cultures and fibroblast cell cultures are separated from each other. Classification due to the confluence character is performed through edge detection, statistical moments and Haralick texture coefficients. The method based on the Haralick texture coefficients delivered the best results. The separation between the cell types is performed through Fourier descriptors, Gabor filtering and through the so-called multiresolution segmentation method. The latter method delivered the highest separability value evaluated with the Fisher's discriminant criterion.

1 Introduction

Automated feature extraction and object recognition are large research areas in the field of image processing and computer vision. There already exist many automated image analysis methods for acquiring numerical information from medical and biological images, especially information concerning cell counting [1],[2] and [3], individual cell analysis and tracking [4], multiple cell analysis and tracking [5], and quantitative measure of cell properties (e.g.size and area) [6]. However, to the best of the author's knowledge, the specific problem of classification of cell cultures due to a degree of cell growth and due to their structure

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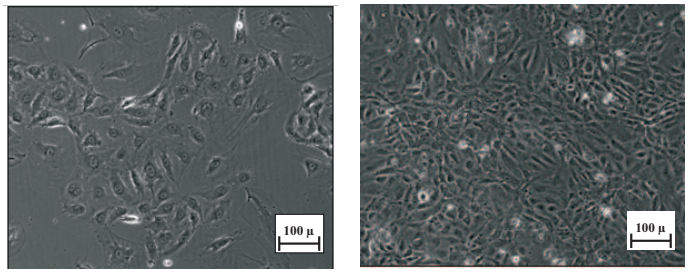
characteristics has not been addressed in the research literature.
Cultivation of human or animal cells and tissues is a widely used technique in many different disciplines ranging from the basic science of cellular and molecular biology to the rapidly evolving field of biotechnology. The list of different cell types, which can now be grown in culture, is quite extensive. If a cell is removed from the original tissue or an organism and placed *in vitro*, then the cell is cultivated in a cell culture. Cell cultures are mainly used as a test object in both basic cell research and pharmaceutical development. The cell cultivation often serves as an alternative for animal tests. In addition, the multiplication of cells is essential for the field of tissue engineering, which is regarded as a promising therapeutic approach in the future.
During the cell growth cells in the cell cultures are regularly examined through a microscope. In the cells cultivation the following parameters are necessary to be regularly checked: whether the cell culture is bacterially contaminated; if the cell culture is confluent (fully grown in a vessel); and whether cells look typical or degenerated. It is obvious that any further manipulation of the cells results from an observer's subjective interpretation.
The need of objectiveness in cell culture observation motivated an idea of image based automated cell culture classification in order to distinguish between cell types. An automated classification process would deliver reproducible and objective results independent of researchers' skills and experiences. As a result, many routine laboratory observations (e.g., counting of cells and detecting a degree of cell growth) would be automated and sped up, thus becoming more effective. Automated cell culture observation could be appreciated in large biological laboratories where many experts are regularly examining microscope images.
The main goal of our research was therefore to develop a method for automated recognition and classification of cell cultures. From the known techniques in the literature 6 image analysis methods for extracting features from images were selected. The subset of features which delivered the best classification results was searched for. The methods were implemented and tested for endothelial and fibroblast cells. The subset of the selected features is able to correctly classify microscope images of the mentioned cell cultures according to the following parameters:

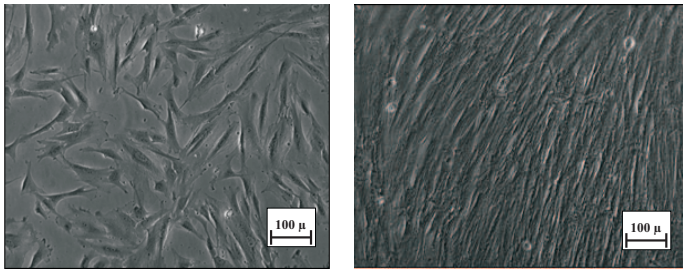
- the degree of growth (confluence character) of a cell culture and
- the morphological features of a cell culture.

Fig. 1- 2 show typical examples of the endothelial and fibroblast cell cultures.
When cells become confluent, totally grown out in a vessel, they need to be passaged in order to meet the essential requirements for their survival and growth. This is why confluence is such an important parameter in cell cultivation. Cells in sub-confluent cultures have enough space to grow and proliferate. Thus, on the images with sub-confluent cultures a uniform background of a vessel, where cells are being cultivated can be observed.



(a) (b)

Fig. 1. A sub-confluent cell culture of HUVEC (Human Umbilical Vein Endothelial Cell) endothelial cells (a) and a confluent (fully grown) cell culture of HUVEC endothelial cells (b). The cells form typical flat, pavement like patterns on the inside of vessels to prevent the blood leakage.



(a) (b)

Fig. 2. A sub-confluent cell culture of fibroblast cells (a) and a confluent cell culture of fibroblast cells (b). The cells form typical fibre like, periodical and oriented patterns in the connective tissue. The fibroblast cells were obtained from stem cells.

2 Materials and methods

Digital images used for the purposes of our experiments were acquired through a transmitted light microscope (Carl Zeiss Axiovert25)using the phase contrast technique [7]. Images were taken with a CCD camera (Zeiss' AxioCam)under magnification of 100 on the microscope. Images were captured in resolution of 1030 x 1300 picture elements (pixels) and exposure time ranging from 50 to 150 ms in order to account for brightness variation. Images were saved in JPEG image format. Altogether 40 images with 10 different exposure times were taken. Images were classified into four classes, each class consisted of 10 images:

- sub-confluent endothelial cell culture;
- sub-confluent fibroblast cell culture;
- confluent endothelial cell culture; and
- confluent fibroblast cell culture.

Our first task was to separate sub-confluent images from confluent ones. For this purpose we searched criteria in the images which would be characteristic enough and numerically measurable to solve this classification problem. It is obvious that in the images of sub-confluent cell cultures some uniform background belonging to a vessel is present. It forms edges with the cell culture. This fact lead us to an idea of counting the number of edges. An edge within an image is a set of connected pixels that lie on the boundary between two regions. Thus, the more edges are on an image the more confluent is a cell culture on it. For detecting discontinuities in intensity values Sobel edge detector was used. By using horizontal and vertical Sobel masks pixels on an image were detected as edge pixels if their value was above experimentally predefined threshold $T = 0.23$. After thinning postprocess, those edge points which belonged to one edge line were merged into an edge line and classified as class *edge line*. Edge lines shorter than 10 pixels were discarded. The remaining edge lines were used to separate between sub-confluent and confluent images.

Texture was the next feature used for classification of sub-confluent and confluent cell culture images. In order to extract texture from the images statistic moments based on the intensity histogram of an image and Haralick texture coefficients [8] were used. Haralick texture coefficients are based on the distribution of intensity values and the position of pixels with equal or nearly equal intensity values.

Our second task was to separate images of confluent endothelial cell cultures from images of confluent fibroblast cell cultures. From Fig. 1(b) one can observe that endothelial cells are rounded and they form rather unoriented pattern whereas fibroblast cells are lengthier and thinner structures Fig. 2(b). They form a pattern which is oriented and periodical. According to the visual structure of the both cell cultures we numerically evaluated their images through the following criteria:

- orientation of the pattern in which cells grow;
- periodicity of the pattern;
- cell's form - rounded or elongated.

The orientation and periodicity "built" from cells were detected through spectral analysis of the texture content using Fourier transform [9], [10] and Gabor filtering [11]. For the purpose of the present problem, Gabor spatial frequency of $\pi/\ 10$ and orientations $n\pi/\ 6$ for n = [0,1 ... 5] were used. Thus, a bank of six Gabor filters was used.

The cell's form was estimated based on the multi-resolution segmentation [12]. Multi-resolution segmentation is a region merging technique. The technique starts with each pixel forming one image object or region. A pair of image objects is then merged into one larger object if they satisfy the local homogeneity criterion. The criterion is determined by the weighted sum of the gray value and shape value. For the present problem of extremely heterogeneous image data, the gray value weight was set to 100 %, since only the shape of image objects was relevant after segmentation. The segmented object primitives of both confluent cell cultures were compared through number of segmented objects, *length*

/ width ratio, compactness and *asymmetry* defined in [13]. In short, the *length / width* ratio is a ratio between the higher and the lower of the eigenvalues of a covariance matrix. *Compactness* is a product of the length and width of an object divided by the number of the object's inner pixels. *Asymmetry* is derived from elliptic approximation and expressed by ratio of the lengths of minor and major axes of the ellipse.

From the six methods implemented for the aims of the image classification, by the help of the Fisher's criterion [14], a combination of the smallest possible subset of features that maximizes the classification success rate was searched for.

3 Results and discussion

The primary objective of the work was to find computationally efficient and reliable methods for separating images of endothelial and fibroblast cell cultures. The "quality" of classification between sub-confluent and confluent images was evaluated with the Fisher's criterion.

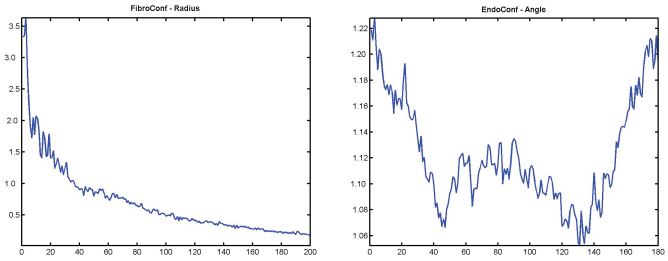
From the first inspection of the images it was observed, that sub-confluent images show high amounts of background area with respect to confluent images. A uniform background was almost nonevident in the confluent images.

	Sub-confluent culture	*Confluent culture*	*Value of the Fisher criterion*
Number of edges	$(30 \pm 5) \cdot 10^2$	$(50 \pm 1) \cdot 10^2$	0.59
Statistical moments	$(30 \pm 5) \cdot 10^{-3}$	$(20 \pm 7) \cdot 10^{-3}$	0.24
Haralick text. coeff.	$(8 \pm 2) \cdot 10^{-4}$	$(2 \pm 1) \cdot 10^{-4}$	4.81

Table 1. Comparison of features which classify images according to the confluence character of the cell cultures. Results are given as mean value ± 2SD.

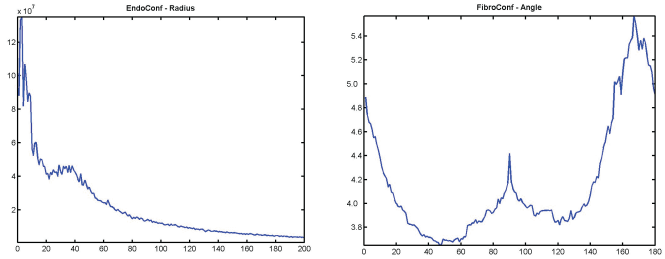
From the Table 1 it is obvious that the number of detected edges is significantly lower on the images with sub-confluent cell cultures. Furthermore, the statistical moments which correspond to a degree of constant background on an image are significantly higher on images with sub-confluent cell cultures. However, the classification task based on the confluence character of the images was the most successfully solved through Haralick texture coefficients. This is because Haralick texture coefficients include information about intensity values and their spatial occurrence on an image. The method with Haralick texture coefficients also delivers the highest value of the Fisher's criterion.

The second classification task based on structural characteristics of cell images was first solved through describing the orientation of periodic or nearly periodic 2-D patterns in an image. From visual inspection of the confluent fibroblast cell culture in Figure 2(b), it can be seen that the pattern is distinctly orientated. In contrast, the pattern of the endothelial cell culture in Figure 1(b) is randomly orientated.



(a) (b)

Fig. 3. The Fourier transform based analysis of confluent endothelial cell culture. The Fourier spectrum is depicted in polar coordinates S(r,θ). The plot of the frequency component S(r)= S(r, $\theta = \theta_0$) of the Fourier spectrum (a). The plot of the angle component $S(\theta)$ = S(θ, $r = r_0$) of the Fourier spectrum (b).



(a) (b)

Fig. 4. The Fourier transform based analysis of confluent fibroblast cell culture. The plot of the frequency component S(r)= S(r, $\theta = \theta_0$) of the Fourier spectrum (a). The plot of the angle component $S(\theta)$ = S(θ, $r = r_0$) of the Fourier spectrum (b).

Fourier analysis numerically proved the results from visual inspection. The angular plot S(θ) in Figure 4(b) shows strong energy components in the region near the origin, 90° and 170° . The peak at nearly 90° corresponds to the strong vertical fibroblast pattern. The peak at 170°, however is associated with a portion

of the constant regions within the image. On the other hand, the angular plot S(θ) of confluent endothelial cells in Figure 3(b) shows the random nature (without distinctive peaks) of its pattern. From the frequency plots S(r) in Figure 3(a) and in Figure 4(a) a slight peak at about 10 units can be viewed. This information served as an input parameter for Haralick texture coefficients and for Gabor filtering.

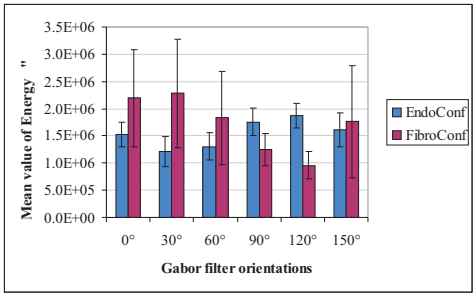
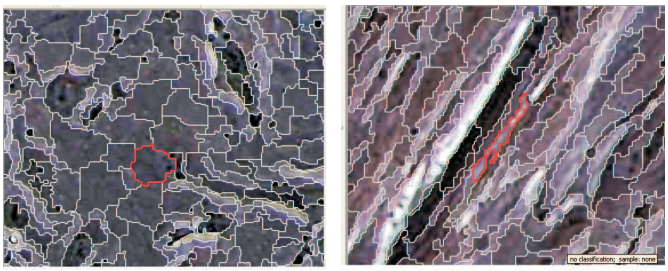


Fig. 5. Mean values of Energy of images after Gabor filtering in different orientations. Vertical lines symbolize the standard deviation.

From results of Gabor filtering on Figure 5 one can observe that values of energy are in general more scattered for images of fibroblast cell cultures. This implies that the direction of texture from fibroblast cells is more distinct than the direction of texture from endothelial cells, which seems random.



(a) (b)

Fig. 6. Segmented image objects after multi-resolution segmentation of a confluent endothelial cell culture (a) and of a confluent fibroblast cell culture (b).

The classification task based on the structural characteristics of cell images is best solved through the features of segmented objects on Figure 6. The first feature compared was the number of segmented objects. From Table 2 it is evident

	Confluent Endothelial culture	*Confluent Fibroblast culture*
Number of segmented objects	$(55 \pm 10) \cdot 10^2$	$(65 \pm 20) \cdot 10^2$
Length / width ratio	$(21 \pm 1) \cdot 10^{-1}$	$(27 \pm 4) \cdot 10^{-1}$
Compactness	$(239 \pm 1) \cdot 10^{-2}$	$(235 \pm 1) \cdot 10^{-2}$
Asymmetry	$(62 \pm 2) \cdot 10^{-2}$	$(72 \pm 2) \cdot 10^{-2}$

Table 2. Results of the statistic comparison of segmented objects. Results are depicted as mean ± 2SD. All results, except the number of segmented objects showed significant difference between endothelial and fibroblast cell cultures.

that the number of segmented object is different for both cell cultures. However, the difference is not significant enough to allow placing a simple threshold for separating the cells patterns. Next, the length / width ratio is used because the visual inspection of both cell patterns shows that fibroblast cell cultures show lengthier and thinner structures than endothelial cell cultures, which show curved and symmetrical shapes. The length / width ratio should be able to distinguish between the object primitives shapes of both cell cultures. The results in the Table 2 numerically prove the assumption beforehand. The ratio is significantly higher for fibroblast cells because the length is longer and the width is shorter than for endothelial cells. Compactness is the similarity of an object to a square or rectangular. Each regular square (or rectangular) has a compactness value of 1. As compactness deviates from 1, shape becomes less square-like. Compactness also delivers significantly different results between both cell groups. Asymmetry is a feature which is very low for symmetric objects (e.g., a circle, a square). The highest value of 1 is for asymmetric objects (e.g. a line). Therefore, asymmetry is a good measure for the assumption that fibroblast object primitives are lengthier and thinner than endothelial objects. Significantly higher asymmetry values were obtained for fibroblast object primitives. In conclusion, the length / width ratio, the asymmetry and compactness are features which most successfully separate images according to the structural characteristics.

4 Conclusion and outlook

In the present work the first step towards the realization of an objective automated classification unit based on images of cell cultures has been made. Images of two cell cultures are successfully classified due to the criteria of confluence and morphological features. For future consideration it is to verify and to extend

the robustness of the proposed methods according to various image qualities and larger data set.

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