Evaluation of HeLa cells growth

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Abstract

In this work, the possibilities for segmentation of cells from their background were tested, combined and improoved. Lot of images with young, adult and mix cells were able to prove the quality of described algorithms. Proper segmentation is one of the main task of image analysis and steps order differ from work to work, depending on input images. Reply for biologicaly given question was looking for in this work, including filtration, details enlargement and segmentation.

1. Introduction

The goal of this study is to analyze the set of images of live HeLa (cancer) cells, growing for experiments focused on cytotoxity in vitro in Laboratory of tissue cultures, Academic and University Center of Nové Hrady. The cells were observed by inverted microscope, using phase contrast. Every 2 minutes was taken one image.

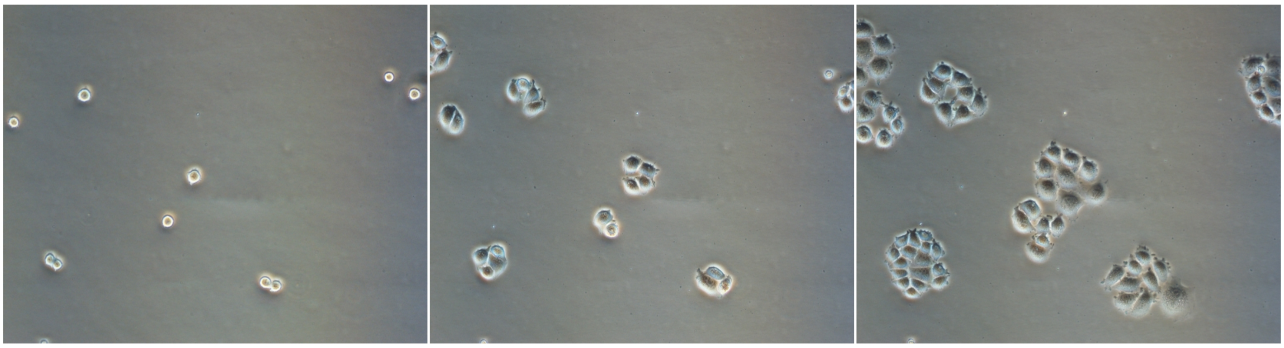

Figure 1: *Examples of growing HeLa cells*

Correct object found depends on plenty of factors, like kind of illumination, shadows, level of presented noise, proper focusing, overlapping to each other or objects dissimilarity to background. Founding process usually starts from simple techniques to more complicated algorithms, until the results are reasonable.

2. Median filtration

To remove the effect of illumination and asperities on background was used Order Statistic Filter which rank neighboring pixels in an attempt to remove low frequency. The output pixels are computed by selecting a neighborhood of pixels around the input point. Then the pixels in the window are ranked according to their intensity. For median filter the middle valued intensity is then assigned as the output value. Large constant regions stay preserved and a thin one pixel line would be removed. Rating beetwen original image and median filter produce image with more uniform background.

3. Appropriate range estimation

Firstly for using the filter on an input image, is important to set the right size of the neighborhood, the filtering window. Any set of points can be divided into statisticaly appropriate number of equidistant intervals using one of these three equations:

i) $k=\sqrt{n}$

ii) $k \leqslant 5 * \log_{10}(n)$

iii) *The Sturges rule:* $k=1+3.3 * \log_{10}(n)$

where *n* is number of points in set and *k* is the count of equidistant intervals [15].

Size of HeLa cell images is $576 * 720 = 414720$ pixels per one image. It gives these possible numbers of intervals:

i) $\sqrt{414720} \approx 645$

ii) $5 * \log_{10}(414720) \approx 28$

ii) $1+3.3 * \log_{10}(414720) \approx 20$

Because HeLa cells are quite small in comparison to the whole image, possibility i) was chosen. In that case, the number is also equal to the number of pixels in the window. Let choose the shape of it. As the basic shape is considered the square, but again according to the objects looked for, the circle shape of window could be better. The filter window was determined as circle with radius *14* pixels : $r=\sqrt{645/\pi} \approx 14$

4. Image border problem

On the all borders of the image only the half circle neighborhood is presented, even only quarter on the corners. As an extrapolation of the missing neighborhood, the size of whole image is on the borders expanded by area of thickness equal to the window radius. Value of extended area is computed as mean value of whole image [1].

Figure 2: *Expanded Image border and Median filtration with and without extended border*

5. Details enlargement

After filtration, still some improovement were tested. Let $p_{i,j,k}$ be intensity value of filtered pixel on coordinates *i, j* and channel *k*. Then $f_x(i,j,k)$ will be the new intensity value of the point $p_{i,j,k}$:

i) *prod:* $f_a(i,j)=\prod_k (p_{i,j,k})$

ii) *square:* $f_b(i,j,k)=p_{i,j,k}^2$

iii) *log:* $f_c(i,j,k)=\sqrt{|\log_{10}(p_{i,j,k}+\frac{1}{512})|}$

In case *prod,* all channels are multiplicated together into grayscale image, where the dark pixels become more darker, and the light become more lighter. Case *square* simply increase the contrast in image. The most complicated is case *log*. Because intensity values are between *0* and *1* , applying logarithm function will rapidly increase the differences between values. The domain range of logarithm function for values from interval *<0,1>* is *(-∞,0>*. For protection against negative infinity, the values were shifted up by small value 1/512. Absolut value operator was then used to convert negative values from logarithm function into positive. And finaly the radical was found, again to change the contrast.

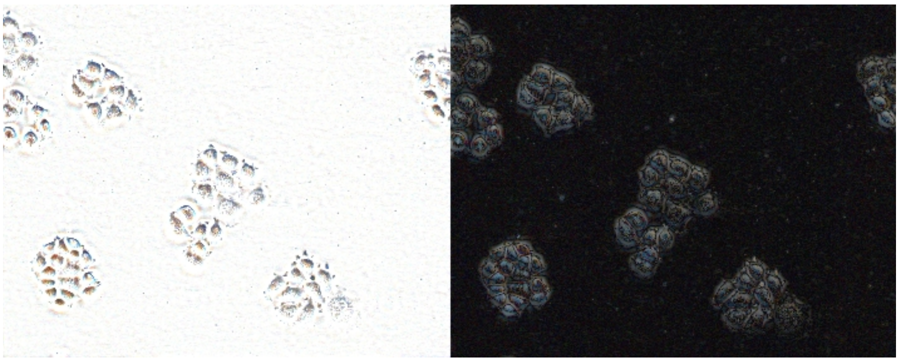

Figure 3: *Rate beetween original and filtered Image and with Logarithmic details enlargement.*

6. Otsu segmentation

Otsu gray level thresholding is a nonparametric method of automatic threshold selection for picture segmentation from intensity histogram *H(p)*. For separating histogram into two classes, the probabilities of of class occurrence and the class mean and the between class variance are computed $\sigma_B^2=\omega_1*(\mu_1-\mu_t)^2+\omega_2*(\mu_2-\mu_t)^2$. The optimal threshold k^* maximazes σ^2_B [2]. The Otsu segmentation was done in all three colour channels (red,green,blue).

7. Binarization

The results are three binary images – one for each channel. Let $p_{i,j,k}$ be the point on coordinates *i,j* and channel *k*. Then the point in final binary image, is $bw_{i,j}$. There are three ways how to make a binary image from three binary channels:

i) *strict:* $bw_{i,j} = \prod_k p_{i,j,k}$

ii) *patient:* $\begin{aligned} bw_{i,j} &= 1 \quad if\,(\sum_k p_{i,j,k}) > 0 \\ &= 0 \quad otherwise \end{aligned}$

iii) *halfway:* $\begin{aligned} bw_{i,j} &= 1 \quad if\,(\sum_k p_{i,j,k}) > 1 \\ &= 0 \quad otherwise \end{aligned}$

In *strict* case, the point that is considered as object in all channels together become object point in final binary image. Some points may be lost, because they have not to be presented in all channels congruously. On the other hand, the *patient* case, allows to all possible points from all channels, to be correctly segmented object points. That can make a lot of fake points. In equilibrium between first two cases is the *halway* one, where only points that are in at least two channels, remain in the final binary image. There is lower level of lost points than in *strict* case, and also lower level of fake points than in *patient* case.

8. Smallest object clearance

To remove objects that passed throught the Otsu segmentation but are not HeLa cells, some morphological operations could be used. The contour of the objects and holes were done by discrete case of Beucher gradient is defined as arithmetic difference between the dilation and the erosion of the image with the structural element B: $g_{SE} = \delta_{SE}(X) - \epsilon_{SE}(X)$ [3].

For all arised lines was computed their length. Simply lines shorter then the average length were deleted. From dilated image were counted all objects and computed their area. Similary like in the Beucher case, the objects with area less then average area, were deleted. But it is still dilated image, that mean all objects are little bit larger then in the started segmentation. Using dual erosion could created bigger holes inside the objects. Better solution, how to shrink the objects is to substract the remaining Beucher gradient lines from remining dilation.

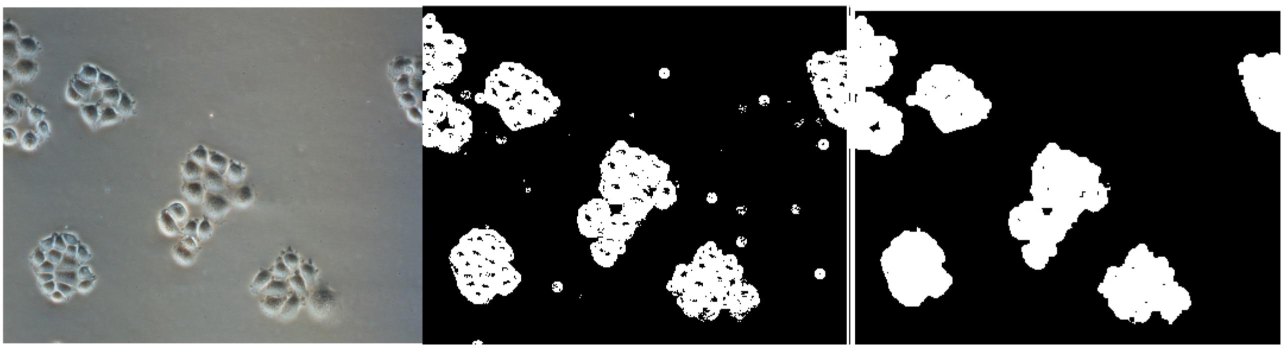

Figure 4: *Original Image, Otsu segmentation and cleared objects.*

9. Growth eavaluation

From cleared binary image percentual appearance of cells in image was computed. This was done for all taken images and the curve of growing was ploted.

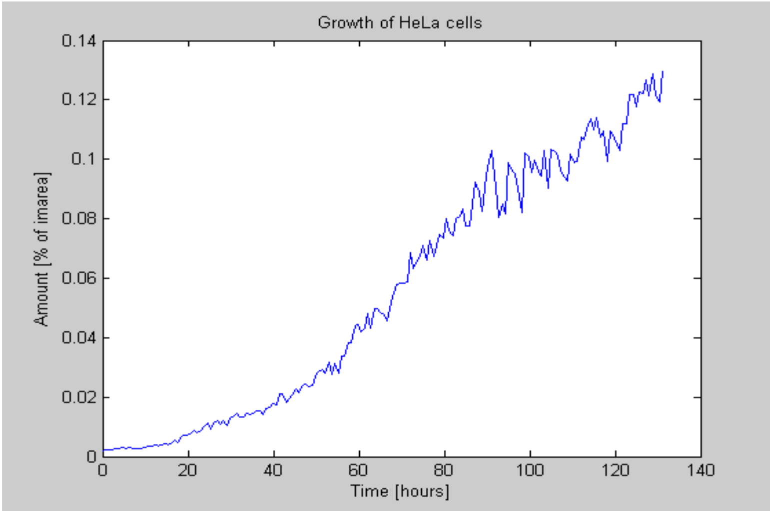

Figure 5: Growth of HeLa cells in time

All methods and algorithms were writen as Matlab m-files.

References

[1] Vaněk J., Urban J., Gardian Z.: „Automated detection of photosystems II in electron microscope photographs", *Technical Computing Prague, p.102,* 2006

[2] Otsu N.: „A Threshold Selection Method from Gray-Level Histogram," *IEEE Trans. on Systems, Man, and Cybernetics SMC-9, pp. 62--66*, 1979

[3] Rivest J.F., Soille P., Beucher S.: „Morphological gradients" , *SPIE "Image Science and Technology"*, 1992.

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