INVESTIGATION OF A COMBINED TEXTURE AND CONTOUR METHOD FOR SEGMENTATION OF LIGHT MICROSCOPY CELL IMAGES

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Abstract

This paper describes a study of the development of a system for the segmentation of cells in order to help to study their behaviour. The new segmentation technique combines a texture based technique with a contour based technique. The paper presents results of both independent methods applied to analyse neutrophil movement. In addition these results are compared with the combined method.

Key Words
Cell movement, segmentation of microscopic images, neutrophils

1. Introduction

Neutrophils are a very important component of the body’s immune system. Neutrophils leave the blood vascular system near the place of a bacterial infection. Then they shift into the direction of the “intruder”. They crawl on the surface of internal organs, and the collagen fibres net to take up the intercellular spaces. After reaching the target, neutrophils kill the “intruder”. Their ability to change place quickly and effectively is one of the important factors influencing the efficiency of the immune system [1].

Thus there is a need to examine neutrophil movement under microscopic observation. If quantitative description of neutrophil behaviour is needed the video sequence with crawling neutrophils can be stored and analysed by computer software. Quantitative description of such complicated behaviour is typically done based on statistical analysis of movement trajectories[1]. The most difficult step of this analysis is segmentation of large numbers of static images. This paper proposes a new semiautomatic method of microscopic image segmentation adjusted to detect active neutrophils on static images using information about previous images in the image sequence.

1.1 Characteristics of crawling neutrophils images

Static images of crawling neutrophils are difficult to segment because of the nature of cell movement and its imaging in brightfield microscopy which is typically used in living cell studies.

Neutrophils in motion develop pseudopodia a delicate sheet-like extension of cytoplasm, approximately 1 micrometer thick. An important feature of the locomotory process is large and broad frontal pseudopodium called lamellopodium. Lamellopodia, with regularly spaced small protrusions resembling fingers along its edge, form temporary contacts with the substrate and lead a cell in its direction [2].

Because a cell is a transparent object its cytoplasm absorbs light to a certain degree. The cell body in light transmission microscopy with bright field optics is visible as a non-uniform shape complex of dark and light spots because of various degree of light absorption by various cell structures, see left column on fig. 1. The cell pseudopodia region is more transparent and homogenous than the cell body but this region is very slightly contrasted with the background. Only slight shifts in mean value of the grey levels are observed in these regions, see black arrows on fig. 1. So there are some distinguishing differences of grey levels and of their range and frequency in various cell regions.

Furthermore the cell presents a large variety of elongated shapes during movement because of developed pseudopodia. Some of these pseudopodia sometimes disappear and appear from image to image in a sequence. This happens because the movement of pseudopodia occurs along the z-axis, perpendicular to the plane, and thus cannot be clearly observed [3].

Observations of cell behaviour are documented by a sequence of images recorded such that cells from one static image to the next one move slightly. Thus it is possible to find the new position of the cell boundary in the nearest neighbourhood of that previous position. The
2. Methods of segmentation

So far effective automatic segmentation of live and not coloured cells have been proposed for microscopic techniques other than bright field (e.g. for interference contrast [4]). Semi-automatic methods have been used to segment complicated images of living cells in brightfield microscopy. One of these techniques was proposed by Korzyńska [1, 5]. This method is based on analysis of the texture and has been used in neutrophil movement analysis. Another method, based on gradient analysis, was proposed by Hoppe et al. [6, 7], and has been used to observe behaviour of cancer cells in culture.

2.1 Texture based technique

The texture based segmentation method (upper line on Fig. 1) divides the image into blocks (upper right path on Fig. 1) to investigate their homogeneity using texture analysis. The statistical features of grey level distribution: the mean value of grey level $m$ and its standard deviation $SD$ (upper middle path on Fig. 1) are calculated for these blocks where we expect new position of cell boundary. The expectations are done according to assumed model of cell movement and cell edge detected in previous step. Two values: mean $m_{ref}$ and standard deviation $SD_{ref}$ of grey levels are quantified for background represented by “reference area”, selected by operator. Blocks with “similar” texture, according to following criteria:

$$\frac{|SD - SD^{ref}|}{SD^{ref}} \geq SD_{thr} \quad \text{or} \quad \frac{|m - m^{ref}|}{m^{ref}} \geq m_{thr}$$

(1)

are assigned to the background texture, otherwise they are assigned to the area of object. These criteria are based on two threshold values selected by operator: $m_{thr}, SD_{thr}$. Next the connectivity of the selected region is examined and the detected region is evaluated [1, 5]. Changes in both threshold and size of block allow adjustment of the criteria and accuracy to individual cells but leads to results that are operator dependent.

2.2 Contour based technique

The contour technique (bottom line on Fig. 1) describes a cell by approximating the location of its boundary using line profiles which emanate from the centre of the cell to the boundary.

The initial placement of vertices is performed on a radial profile (on Fig. 1 right bottom path) from a position selected by the operator. The Prewitt gradient (on Fig. 1 middle bottom path) and cumulative function of this gradient along these profiles are calculated. The vertices are placed on the outer boundary, where the cumulative gradient along the profile reaches saturation. These points are found based on points chosen by the operator with an initial threshold value $th^{in}$ adjusted to strength of the gradient along each profile by the function:

$$th = th^{in} + (0.1 - \frac{grad_{max}}{10})$$

(2)

where $grad_{max}$ is the maximum value of each globally normalized cumulative gradient.
Once an approximate boundary has been found, the spline vertices are re-sampled and an orthogonal profile is calculated. Using the orthogonal profile, the position of vertices are refined and located at the maximum gradient, orthogonally to the initial boundary [6, 7].

This technique is also dependent on operator decisions because the operator indicates the centre of the cell and chooses the initial threshold value.

2.3 Results from both techniques

It was found that segmentation results for both techniques vary according to the cell boundary. When cells with circled shape move the both techniques results of segmentations are located close to the cell boundary and to each other.

If the cell develops or retract pseudopodia, the segmentation results of both techniques differ. This is because the gradient method is not sensitive to small changes in grey level and reduces regions where strong gradients are absent - Fig. 5 b.

The texture analysis technique leads to over-estimation of cell area – Fig. 2 and 5 c. This over-estimation of cell region is dependent on the texture block size. For blocks with a size up to 5x5 an over-estimation of cell area occurs in up to 20% of the detected areas. If the block size is greater than 5x5 the over estimation is greater than 20%. The best segmentation results are observed for a block size of 3x3 with slight overestimation of the area. For the upper cell shown in Fig. 5 the over-estimation is 12%. To segment cells with very weak gradient and pseudopodia, the size of the texture blocks needs to be larger – Fig. 2. It is appeared that the size of blocks should be approximately half of the size of the gap where there are not well defined gradients; however over-estimation of the area is related to the gap size.

To examine cell movement, the centres of gravity of the lamina cell model [3] are calculated based on the detected edges; from this the cell movement trajectory is constructed. The over-estimation of the cell area may not necessarily have a large affect on the position of the centre of the cell because the extra area is homegeniously distributed around the cell. However if an important part of cell, for example leading lamella, is inapproprately segmented the position of the cell centre can be significantly different.

3. Hybrid method of segmentation

We propose a new hybrid segmentation technique that integrates both techniques – contour based and texture based segmentation methods. This method is designed to reduce weaknesses of each method and to use their strengths.

In Fig. 2, the texture segmentation result is overlayed onto the original cell image. The cell image is clearly over segmented; however, the expanding pseudopodia regions (arrows) have been included in the segmentation. Information about the overestimated area is useful as a first estimation of a cell outline.

Feature vectors are generated from the radial profile as shown in Fig. 1. We use the information from the texture space $I_{tex}$ (for block size 3x3, 5x5, 7x7 and so on; the mean value and the standard deviation thresholded binary image) and gradient space $I_{grad}$ (3x3 Prewitt filter). The texture method is performed on the full (1393x1040) resolution images and the gradient calculations have been performed on decreased resolution (half) and are smoothed by a Gaussian filter. Information from both feature vectors is used by a linear combination of features from both feature spaces:

$$I = (1-\alpha)I_{grad} + \alpha I_{grad}I_{tex}$$

where $\alpha$ is used as a parameter to set the weighting of the texture segmentation. Fig. 3 shows the feature space of the original gradient image (a) and the new combined feature space (b) which is used to find vertex positions. Each column describes a single radial profile line. Both images have been equalised for display purposes only in order to highlight that the edge region is better distinguishable after both feature spaces have been combined.

This leads to a more accurate estimation of inflexion point of the cumulative gradient function. The cumulative of the column feature vectors is plotted in the Fig. 4. In pseudopodia regions, where the previous gradient method did not find a point of inflexion properly, the new combined technique is improved.

This technique is less dependent on operator decisions then the gradient technique because the centre of region detected by texture based segmentation technique and previous image centre point are used to estimate central point of the cell on actually segmented image.
Fig. 3. Space of line profiles
(a) gradient feature space
(b) feature space of linear combination with $\alpha=0.5$

4. Analysis of effectiveness of combined method

The texture and gradient based techniques work on very different principles. The contour based technique results in a closer fit to the cell boundary wherever a strong enough gradient is detectable. However, expanding cellular regions often express a low contrast and weak gradient. These areas are likely to be poorly segmented by a contour method even though the gradient feature space is normalised for each profile line and adapts to local fluctuations along the contour.

Texture based techniques intrinsically reduce resolution and thus result in a coarser segmentation. In order to increase sensitivity for the pseudopodia regions, the threshold value to distinguish between background and cellular areas is set close to the mean and the standard deviation of the background. This results in false classifications and over segmentation at the boundary region.

It seems that the weaknesses and strengths of both techniques are complementary, and thus a new combined method should lead to more accurate segmentation.

In Fig. 5, there are two cells: first (upper line) cell with small pseudopodium and second (bottom line) with large pseudopodia region (arrow on middle column d). Furthermore there are results of combined method (a), gradient (b) and texture (c) techniques of segmentation and images showing difference of gradient and texture results (e), texture and hybrid results (f) and gradient and combined method results (g).

The combined method of segmentation puts the contour somewhere between contours detected by the gradient and the texture based techniques. In regions with strong gradients, the new method contour is located close to the gradient method contour; however in regions of pseudopodia the combined method is close to the texture results. On the difference images (f, g) the absolute difference between texture and combined method results are larger than between the gradient and combined methods. So the position of the combined method cell contour is closer to the contour found by the gradient method than the texture method.

Fig. 4. Cumulative gradient plot with threshold indicated at $T=0.9$ (---). The cell image shows the result of new combined segmentation method
Fig. 5. Cells with small (upper line) and large (bottom line) pseudopodium region (▶) segmentation result:

- a. The hybrid method result for $T=0.88$ and $T=0.8$
- b. The gradient techniques result for $th^{int}=0.8$
- c. The texture techniques result for: block size 3, $m_{thr}=0.6$, $SD_{thr}=0.1$ and block size 5 $m_{thr}=0.5$, $SD_{thr}=0.12$
- d. The texture segmentation result overlayed on image
- e. The difference between texture and gradient techniques
- f. The difference between texture techniques and combined method
- g. The difference between gradient techniques and combined method

The mean value and the standard deviation of distances between the centres of gravity were evaluated based on the combined method of segmentation and both individual techniques. The mean distance between the cell centres in the texture and combined methods was $5\pm 4$ pixels for all quantified images. However in the group of closely located trajectory fragments, the mean distance was $4\pm 1$ pixels and in the group of far located trajectory fragments $7\pm 5$ pixels. The distance between the centres of the cell in the gradient and combined methods was smaller: $3\pm 2$ pixels. In the group of closely located trajectory fragments the mean distance was only $2\pm 1$, while in the group of far located trajectory fragments: $3\pm 2$. So the positions of cell centres calculated by the new combined method were located closer to the position obtained with the gradient technique, than to the texture one. This agrees with observations of contour location. Mean and its standard deviation of cell area and cell smoothed perimeter are shown in table 1.

Based on detailed examination it was found that there are cells rounded or elongated and without pseudopodia in fragments where both trajectories are close. In the second group with a large distance between trajectory the cells’ shape appeared more complex with pseudopodia.

### 4.1 Comparison of both segmentation methods with results of combined method

The movement of neutrophils was monitored in the “Cells Behaviour Monitoring System” [1, 3]. Two sequences of images, one with 3 cells and the second with 5 cells were captured on computer. These sequences contain 120 images recorded neutrophil behaviour during one minute. The movement trajectories for three randomly chosen cells were constructed using the centre of gravity calculated using the texture, the gradient techniques and the combined methods. Six parts of the cell movement were selected, for further detailed analysis: three short subsequences where constructed trajectories were located closely were treated as first group and three subsequences where trajectories located far one from the other were treated as the second group. For both groups the following values were compared: segmented area, length of smooth contour and position of the centre of gravity [8].

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Table 1 shows that the mean area detected in the tested population obtained from the new combined method is larger than the gradient method and smaller than the texture method. Lengths of smoothed perimeter results are in good agreement with the results of area calculation. This implies that objects, detected with the combined method, are located and sized somewhere between that obtained by the both individual techniques.
Based on these results it was concluded that the results of the new method were generally closer to the gradient technique results of segmentation than to the texture technique. The gradient technique in the new proposed combined method had advantages of accuracy in edge and smoother contour detection.

5. Conclusions

The new combined segmentation method integrates advantages of two distinct segmentation approaches: – contour method by detection of discontinuity in grey level and - area finding by detection of region homogeneity. For the cells with pseudopodia the segmentation results are dependent on the sensitivity of the texture method but the boundary position depends on gradient method.

The new combined method produces segmentation results, which are in a good agreement with the individual techniques for centre of gravity and the length of the smoothed contour. The results of the segmented area seem to be better than from the both individual techniques separately.

The results from the comparison of the techniques clearly show potential advantages of the combined method.

6. Acknowledgement

We are grateful for support received from the British Council.

Table 1. Area and perimeter of segmentation analysis in the tested population and in two subpopulations: first with three (a, b and c) closely located trajectories fragments and next three (d, e and f) far located trajectories fragments

<table>
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<tr>
<th></th>
<th>gradient technique</th>
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<th>combined method</th>
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<td>far located</td>
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<td>d. 1.4795±554</td>
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<td>e. 1.0724±457</td>
<td>b. 1.3824±338</td>
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<td>f. 1.2006±787</td>
<td>c. 1.1330±521</td>
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<td>d. 459±9</td>
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<td>e. 369±8</td>
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References


