STEM CELL MICROSCOPIC IMAGE SEGMENTATION USING SUPERVISED NORMALIZED CUTS

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ABSTRACT

A vast amount of toxicological data can be obtained from feature analysis of cells treated *in vitro*. However, this requires microscopic image segmentation of cells. To this end, we propose a new strategy, namely *Supervised Normalized Cut Segmentation* (SNCS), to segment cells that partially overlap and have a large amount of curved edges. SNCS approach is a machine learning based method, where loosely annotated images are used first to train and optimise parameters, and then the optimal parameters are inserted into a *Normalized Cut* segmentation process. Furthermore, we compare our segmentation results using SNCS to another four classical and two state-of-the-art methods. The overall experimental result shows the usefulness and effectiveness of our method over the six comparison methods.

Index Terms— Image Segmentation, Stem Cells, Machine Learning, Supervised Normalized Cut

1. INTRODUCTION

Modern toxicology uses changes in individual cells as endpoint to determine and quantify adverse effects. One approach is to use human migratory neural crest cells, a transient fetal stem cell type, to study developmental toxicity by investigating changes in cellular features observable by microscopy. In previous experiments, it was observed that these cells change their morphology upon treatment with chemicals and different chemicals induce different cell shapes. We hypothesize that if two compounds induce the same cell shape, they interfere with the same biological process. Therefore, we segment microscopic images of these cells in a first step, then analyze cellular features in a later step.

As these cells are grown in relatively dense culture, they are in contact with each other and have a lot of curved edges. This makes their segmentation very difficult. Existing segmentation methods such as edge detection or watershed transformation could segment the main bodies of cells well, but lose efficacy when the cells are dense or have many curved edges. For example, edge detection algorithms can localise edges, but when the density of objects is very high, many adjacent edges link together and lead to a wrong segmentation result (see Fig. 2(b)). Because watershed usually merges many unrelated regions and easily results in an over-segmentation of the image, it cannot exactly capture the curved edges of cells under biological treatment (see Fig. 2(c)).

Due to these two problems mentioned above, we establish a machine learning based segmentation method, the *Supervised Normalized Cut Segmentation* (SNCS), which builds a data-driven system using input examples, leading to a more accurate segmentation result. This method enables us to segment cells even if they touch each other and have many curved edges. The SNCS approach is constituted by a training and segmentation step. In the first step, training images are first manually segmented, then they are used in a supervised learning framework for optimising parameters for the segmentation. In the second step, the trained parameters are inserted into a *Normalized Cut* (NC) approach [1] to obtain the segmentation results for test images.

2. RELATED WORK

2.1. Cell Segmentation: Image segmentation methods have been applied to different kinds of cell images. Segmentation and classification of cancer cells was discussed in [2] and [3]. In [4] and [5], image segmentation methods are presented for the blood cell analysis. Other applications include segmenation of muscle cells [6] and brain cells [7]. However, there are very less work on stem cell segmentation, and the challenges of stem cell segmentation result from cell overlapping and their curved edges [8].

2.2. Segmentation Approaches: Medical image segmentation methods are mainly categorized into 'boundary based', 'region based' and 'model based' [9]. Boundary based methods are based on the edges between different regions, which are fast to compute and do not need priori information about image contents. For example, many edge detection algorithm-

s (e.g. Sobel method) are based on the change of the gradient of gray levels [10]. In [11] a new contour detection and hierarchical image segmentation method is proposed, where local and global information is combined to improve the segmentation performance of the basic edge detection methods. However, a main disadvantage of boundary based methods is that they have a low efficiency for segmenting dense objects.

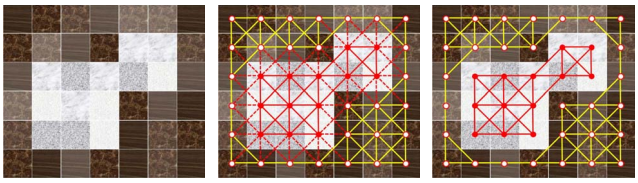
Region based segmentation methods are based on the similarities between regions, including region growing, watershed and NC methods. The region growing method segments an image by connecting neighboring pixels based on a similarity criterion [12]. The watershed algorithm uses image morphology to segment images, and can recognise different regions in an image by using a morphological watershed transformation [7]. The above three methods exhibit two problems: Images of dense objects are over segmented, and all methods have a low efficiency for capturing cells with curved edges. In contrast, the NC method computes a similarity matrix based on intervening contours and segments the image according to a distance criterion [1]. It shows a promising ability to solve the over-segmentation and curved edge problems.

Markov Random Field (MRF) based segmentation method is a model based segmentation approach, which is a natural way to incorporate spatial correlations into a segmentation process [13]. The MRF is a stochastic process that specifies the local characteristics of an image, and it is combined with the given data to reconstruct the true image.

Among all the methods mentioned above, the NC method can overcome the over-segmentation and curved edge problems robustly. However, NC is an unsupervised approach, which does not use any priori information of cell shapes, resulting in a high possibility of great deviation within the segmentation results. To reduce the risk of such deviation, we propose the SNCS approach within a supervised learning framework [14], where loosely annotated images are used for training and optimising parameters of the NC approach. Hence, this supervised approach further improves the segmentation performance of the NC method, leading to an efficient method for stem cell segmentation.

3. SEGMENTATION METHOD

3.1. Fundamentals of SNCS: SNCS is a graph based segmentation method which divides an image according to some criterion. In graph theory, a graph is represented as $G = (V, E)$, where V denotes nodes and E denotes edges connecting the nodes [15]. In image analysis, an image can be regarded as a graph, where V represents a set of all pixels as shown in Fig. 1(a), and E denotes a set of all edges connecting the pixels as shown in Fig. 1(b). G can be divided into two separate sets A and B, $A \cup B = V$, $A \cap B = \emptyset$, by simply removing edges connecting the two parts as shown in Fig. 1(c).



(a) (b) (c)

Fig. 1. SNCS segmentation process (a) Original image. (b) Bipartite graph. (c) Segmentation results.

In graph theory, the above process is called a 'cut':

$$C(A, B) = \sum_{u \in A, v \in B} w(u, v) \tag{1}$$

where $C(A, B)$ represents the cut of A and B, and $w(u, v)$ denotes the weight of the edge between u and v. The optimal segmentation of a graph is the one that minimises $C(A, B)$, which is called the 'minimum cut' of a graph [16].

However, the minimum cut criteria tends to cut small sets of isolated nodes in the graph [16]. To avoid this unnatural bias for segmentation, the SNCS criteria is defined following [1]:

$$N(A, B) = C(A, B)/O(A, V) + C(A, B)/O(B, V) \tag{2}$$

where $O(A, V)$ and $O(B, V)$ are the total weights of edges connecting pixels in A and B to all pixels in the graph, respectively. Under this definition, because $C(A, B)$ is a large percentage of both $O(A, V)$ and $O(B, V)$ for the small isolated nodes, the cutting bias problem is effectively solved.

For a given graph $G = (V, E)$, the optimal partition is computed by [1]:

$$\min_x N(x) = \min_y [y^T (P - Q)y]/(y^T P y) \tag{3}$$

$$(P - Q)y = \lambda P y \tag{4}$$

$$P^{-1/2}(P - Q)P^{-1/2} z = \lambda z \tag{5}$$

where x is an indicator vector, y is a non-zero vector, P denotes the total connections of the node, Q denotes the weight matrix of the connections, λ is the eigenvalue and $z = p^{-1/2} y$. Therefore, computing the optimal partition can be regarded as a problem of seeking the eigenvectors with the eigenvalues of Eq. (5).

3.2. Initialisation of SNCS: When segmenting an image, the SNCS algorithm is initialised by an unsupervised clustering process, consisting of the following five steps [1]: (I) Given a weighted graph and a set of features to measure similarity, then we define the weight on each edge and summarize the information into Q and P. The weight on the edge should reflect the similarity between each two pixels. For a gray-scale image of a cell, we use the brightness value of the pixels

K and their spatial location R to define the graph edge weight connecting two nodes i and j as [1]:

$$w_{ij} = e^{-\|K_i - K_j\|_2^2/\sigma_1^2} * \begin{cases} e^{-\|R_i - R_j\|_2^2/\sigma_2^2} & , \quad \text{if} \quad \|R_i - R_j\|_2 < r \\ 0 \quad , & \text{otherwise} \end{cases} \tag{6}$$

where σ_1 and σ_2 are empirically set constants in an unsupervised NC system, and r is a predefined threshold. (II) Solve $(P-Q)x = \lambda Px$ for eigenvectors with the smallest eigenvalues. (III) Use the eigenvector with the second smallest eigenvalue to bipartition the image by finding the splitting point, so that $N(A, B)$ is minimised. (IV) Decide whether the current segmentation should be subdivided by checking the stability of the cut according to an empirical value σ_3 which is prespecified by Eq. (7). (V) Repartition the segmented parts if it is necessary.

$$\sigma_3 = \min(H(\lambda))/\max(H(\lambda)) \tag{7}$$

where H represents the histogram of the eigenvector values and σ_3 is the ratio between the minimum and maximum values.

3.3. Training and Optimisation of SNCS: In the above initialisation step, SNCS relies on an unsupervised segmentation process, where the parameters σ_1 and σ_2 in Eq. (6), and σ_3 in step (IV) are predefined empirically. There tuning is key to achieve high-quality partitions. Thus, the SNCS approach optimise the parameters using training images. Although meticulously manual annotation is a difficult and time-consuming work, we only use training images which are loosely annotated, so the total effort involved in the training process is reasonable (each cell costs about one minute to segment). Noticing that the proposed approach is an improved NC which is implemented in semi-supervised fashion rather than in a fully supervised fashion, the annotation work is relatively simple. First, training images are manually annotated by biologists according to their professional knowledge. Then, the parameters are optimised using the training images in a supervised learning framework as follows: First, we define an object function as follows

$$S(\hat{A}, \hat{B}) = \text{argmax}_{A,B}[-N(A, B) + M(A, B)] \tag{8}$$

where $\hat{A}$ and $\hat{B}$ are the corresponding optimal pixel sets for A and B, $S(\hat{A}, \hat{B})$ denotes the final segmentation result of the image, $N(A, B)$ is the SNCS criteria in Eq. (2), and $M(A, B)$ is the similarity between A and B, which is defined as the Euclidean distance between their pixel brightness values. Second, we initialise the parameters σ_1, σ_2 and σ_3 with priori knowledge. Thirdly, we implement the SNCS segmentation over all the training images using the initial parameters. Finally, we calculate the object function $S(\hat{A}, \hat{B})$ with respect to parameters, where a global convergence approach is used to guarantee that iterations converge to an optimal solution. If $S(\hat{A}, \hat{B})$ is not convergent, then the parameters returns to the previous step. We execute this process recursively until $S(\hat{A}, \hat{B})$ gets to a maximal value.

4. EXPERIMENTAL RESULTS

4.1. Experimental Setting: We use 81 microscopic images for test, including approximately 6000 cells. Additional 81 corresponding nucleus images are used to count the actual segments of the image. In order to implement the proposed SNCS method, we use 28 ground truth images including about 1000 cells to train the system for optimising the parameters. The parameters of SNCS are initialised according to the setting of NC in [1]. Furthermore, to compare the segmentation performance of SNCS, we tested six other segmentation approaches, including four classical methods: Sobel edge detection [10], watershed transformation [7], MRF [13] and region growing [12], and two state-of-the-art methods: contour detection [11] and NC [1] methods. Finally, two visible and four numerical comparisons are provided for evaluating the segmentation performance of SNCS using the ground truth images. In our experiments, the number of the training and testing cell images is relatively small. As the proposed method is a supervised method, the performance is closely related to the number and the variety of the training data. When performing the method in a large scale setting with few hundreds to thousands of cells with vastly varying morphology, a large number of ground truth images including various cells are required.

4.2. Experimental Results and Evaluation: Some segmented cells with dense nucleus are shown in Fig. 2. When a segmented region (in colours) contains a nucleus (in black), it has a higher possibility to be correctly segmented. From Fig. 2 we can find that watershed method has the best performance to detect the regions of different cell types. Flowing watershed transformation, SNCS, NC, region growing and contour detection have similar results. MRF based segmentation methods obtain the worst results. This comparison shows that SNCS is robust to segment stem cells when the microscopic images show densely distributed cells.

Fig. 3 shows the comparison of segmented regions (in blue outlines) and the original cells by coincident areas. If the segmentation is more accurate, the outlines are more close to the actual curved edges of the cells.

From Fig. 3, we can find that SNCS and NC obtain the best performance to describe the complicated and curved edges of cells. Sobel edge detection and contour detection have the second best performance. Lastly, watershed and region growing have similar worse results. MRF based segmentation method obtains the worst results, but the details of the cell images are most similar to the ground truth image. This comparison proves the usefulness of SNCS for solv-

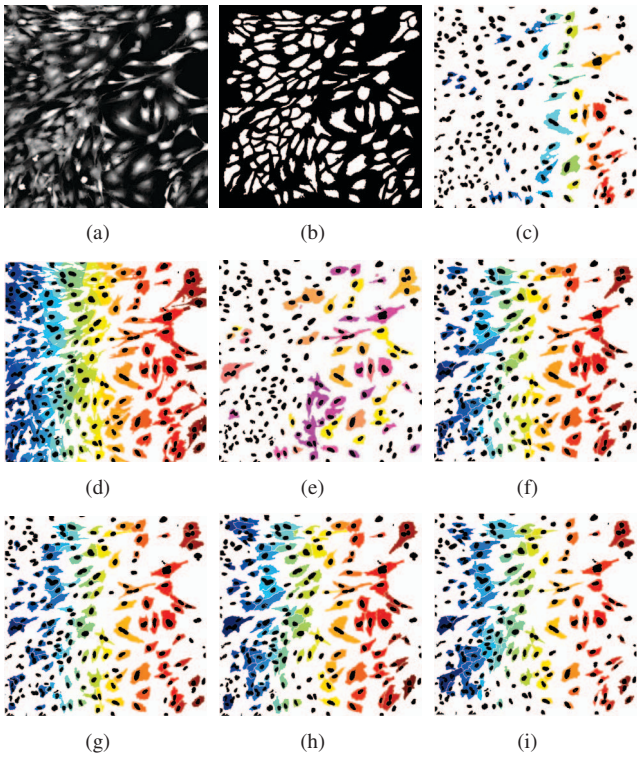


Fig. 2. Coloured results with nucleus of seven different segment methods. (a) Original stem cell image. (b) Ground truth. (c) Sobel Edge Detection. (d) Watershed. (e) MRF. (f) Contour Detection. (g) Region Growing. (h) NC. (i) SNCS.

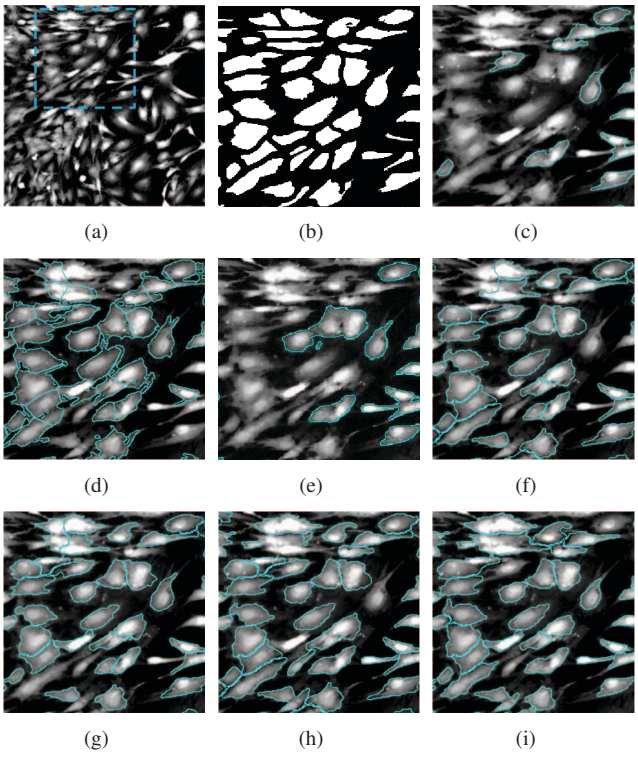


Fig. 3. Outline results of seven different segment methods. (a) Original stem cell image. (b) Ground truth. (c) Sobel Edge Detection. (d) Watershed. (e) MRF. (f) Contour Detection. (g) Region Growing. (h) NC. (i) SNCS. For a detailed observation, a part of (a) in a blue boundingbox is zoomed in.

ing the segmentation problem for cells with curved edges. Furthermore, we use four numerical measures, similarity, sensitivity, specificity [17] and RA (see below), to evaluate the segmentation results. Similarity indicates the overall consistency between segmented and ground truth images. It is a value in $[0, 1]$. If the similarity is close to one, segmentation is very similar to the ground truth. Sensitivity and specificity represent the consistency of foreground and background, respectively. RA is the ration between obtained segmentation results and the existing number of cells, and it is defined as

$$\text{RA} = \frac{\text{number of the segments}}{\text{number of the actual nucleus}} \times 100\% \qquad (9)$$

The segmentation result is more accurate, if RA is close to 100%. The segmentation method is under-segmentation, if $\text{RA} < 100\%$, and over-segmentation, if $\text{RA} > 100\%$. A numerical comparison is shown in Table 1.

Table 1 shows that SNCS obtains the highest similarity, the third highest sensitivity, the best specificity, and the best RA. The RA of SNCS is closest to 100%, demonstrating that the proposed method can detect cells accurately in an image with dense cells. Furthermore, the overall result of these four measures shows that the SNCS outperforms other methods. Thus, we prove that SNCS outperforms traditional NC due to its machine learning approach.

Table 1. Numerical comparison of segmentation results (in $[\%]$). The best results are in bold.

Methods	Similarity	Sensitivity	Specificity	RA
Sobel Edge Detection	90.8	**97.6**	66.7	49.8
Watershed	92.3	33.3	81.0	140.6
MRF	85.1	42.7	73.5	25.9
Contour Detection	**92.4**	40.0	89.3	128.8
Region Growing	**92.4**	21.2	95.2	121.7
NC	92.3	52.1	95.2	118.3
SNCS	**92.4**	50.3	**96.0**	**107.8**

5. CONCLUSION AND FUTURE WORK

In this paper we propose a SNCS method for segmenting individual stem cells from an image, which takes advantage of the justice of clustering criterion in NC algorithm and the robustness of supervised learning processes. The experimental results show a good performance of our method. In the future, we will use the segmentation results of SNCS in a clustering process to aid taxonomic tasks in relation to stem cells [18]. Additionally, we will deal with annotating ground truth images and segmenting cells in a large scale setting.

6. REFERENCES

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