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A Confident Scale-Space Shape Representation Framework for Cell Migration Detection

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SUMMARY

Automated segmentation of time-lapse images is a method to facilitate the understanding of the intricate biological progression, e.g., cancer cell migration. To address this problem, we introduce a shape representation enhancement over popular snake models in the context of confident scale-space such that a higher level of interpretation can hopefully be achieved. Our proposed system consists of a hierarchical analytic framework including feedback loops, self-adaptive and demand-adaptive adjustment, incorporating a steerable boundary detail term constraint based on multiscale B-spline interpolation. To minimize the noise interference inherited from microscopy acquisition, the coarse boundary derived from the initial segmentation with refined watershed line is coupled with microscopy compensation using the mean shift filtering. A progressive approximation is applied to achieve represented as a balance between a relief function of watershed algorithm and local minima concerning multi-scale optimality, convergence, and robust constraints. Experimental results show that the proposed method overcomes problems with spurious branches, arbitrary gaps, low contrast boundaries and low signal-to-noise ratio. The proposed system has the potential to serve as an automated data processing tool for cell migration applications.

Keywords

cellular image segmentation; 3T3 cell; time-lapse microscopy; snake model; multiscale detail detection; mean shift filtering

1 INTRODUCTION

The research based on cellular images is important in investigating disease mechanisms and signaling pathways at the cellular and molecular biology levels. For example, high resolution images of cancer cells can be used to determine the progression of cancer cell migration, indicating the invasion of cancer cells and cancer metastases (Mathew *et al.*, 1997). Today, automated fluorescence microscopy is increasingly popular for the acquisition

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of time-lapse cellular images for such studies. The automated microscopy techniques however generated large amounts of image datasets such that traditional manual analysis methods are not scalable to handle the datasets. Image analysis becomes a bottleneck in time-lapse bioimaging. Automated and fully adapted analysis methods are being actively researched.

A variety of approaches have been proposed in the literature, with image segmentation as one of the most critical issues. Image segmentation is devoted to yield a partitioning of the image into disjoint regions with a uniform contour of shape, by achieving a balance between adherence to the possible noisy and smooth boundary. The geometry of images comprises edges, ridges, corners, blobs, and junctions which are usually characterized of differential operators. As an important geometric feature of shape representation, edges are usually detected by examining the local maxima. There exist two major approaches for edge-based image segmentation: the watershed algorithm from mathematical morphology (Roerdink & Meijster, 2000) and the minimization of certain energy function (Williams & Shah, 1990).

The watershed approaches are based on an immersion process analogy with edge evidence derived from the morphological gradient. Owing to its advantages such as the proper operation of gaps and the orientation of the boundaries (Vincent & Soille, 1991), the watershed algorithm has been used in many application fields of image processing (Bonnet, 1998) (Boselli *et al.*, 1999) (Cremona *et al.*, 2000). Unfortunately, the watershed transform is difficult to impose *a priori* information, e.g., smoothness on the watershed lines. Because of the sensitivity to noise, over-segmentation, and poor detection of thin structures, the watershed transform becomes unacceptable for our application of cell migration.

Compared to the watershed transform, the snake as an energy-minimizing spline guided by data adherence and smoothness constraints, takes a different approach as active contour models (Kass *et al.*, 1987). The snake-based contour is consisted of curves which move under the influence of internal forces coming from the curve itself and external constraint forces computed from the image data (Derraz *et al.*, 2004). Snake-based methods have serious drawbacks too. The initial contours have to be close to the actual boundaries otherwise the snakes will likely converge to the wrong object or non-significant edges. On the other hand, snakes have difficulties in tracing the boundary cavities.

Several hybrid approaches have also been proposed to extract boundaries in bioimaging. Intuitively, watersnakes as an energy-driven watershed segmentation is proposed to exert the smoothness control based on energy minimization (Nguyen *et al.*, 2003). Fok *et al.* (1996) introduced a snake-based method to extract axons boundaries. Klemencic *et al.* (1998) used another snake-based method to analyze muscle fiber images. Ray *et al.* (2002) proposed an active contour based technique with shape and size constraints to track leukocytes in vivo. Wahlby *et al.* (2004) used a combined method to retrieve cell nuclei from fluorescence microscopy images. A seeded PDE based segmentation of nuclei and cell is used by Solorzano *et al.* (2001). Another approach of medical image edge detection based on mathematical morphology has been proposed to recognize human organs and applied to detect the edges of lungs CT images (Zhao *et al.*, 2005).

Although the approaches mentioned above have certain advantages and applications, as to the analysis of automated microscopy images of cell migration, little of those can provide us with satisfactory results. In this paper, we attempt to utilize a progressive approximation which represents a balance between a relief function of watershed algorithm and improved snake approach concerning multi-scale information to extract actual boundaries of target cell. The approach described in this paper is a fully automated and fast method. It can deal with images of low SNR and fuzzy, irregular, and ruffling cell boundaries, while preserving

details of regions of interest. The remainder of the paper is organized as follows. Section 2.1 describes the initial segmentation that supplies a coarse boundary. Section 2.2 describes the microscopy compensation aims to remove the specific adjoin noise. Section 2.3 and Section 2.4 presents the detail confidence calculation and preservation. Experimental results are provided in Section 3, and Section 4 provides the conclusions.

2 METHOD

As described above, most of the aforementioned approaches follow a straightforward processing track without a feedback loop. In order to improve segmentation results, we define a hierarchical analytic representation framework which includes feedbacks and self- and demand-adaptive analysis and adjustment shown in Fig. 1. Our approach can be divided into three steps:

1. Initial segmentation step including watershed algorithm and post-processing procedure. It is designed using *a priori* information, such as average surface that is covered by cell area in the image, to prevent over-segmentation and impose the constraints of features. With such *a priori* information considered, we can reduce the influence caused by the variation of environmental conditions during image acquisition. The initial segmentation mainly focuses on how to form a coarse cell boundary to be used in subsequent processing.
2. Microscopy compensation using mean shift algorithm. The microscopy compensation is dedicated to clustering the image-dependent noise and preserving the edge through mean shift filtering. This technique can especially reduce the influence of the noise imposed by microscopy imaging represented in the form of some white regions near the cell boundary while minimizing the fading of weak boundaries. With this compensation, we can identify and locate these inevitable white regions and eventually remove them using erosion algorithm. This step makes it possible to acquire a set of boundary pixels which is close enough to the real boundary and to engender the input boundary set for more precise boundary detection.
3. Detail preservation with confidence calculation. Considering that most tenuous gaps and spurious branches along the cell boundary derived from anisotropic modality, we employ B-spline function to reconstruct cell boundary and use multi-scale based technique to elegantly identify the area which could be the parts that the biologist would pay more attention to. The high-level boundary shape could be locally and iteratively approximated to manageable regions-of-interest of finer details, by an interactive mechanism using a low-level contour model. Taking the cell filopodias as an example, the number and the shape of filopodias are important to cell migration study, and we process the pixels near the filopodias with care. This step is designed to obtain a tighter and smoother boundary which is very close to the actual one while maintaining boundary details.

2.1 Initial segmentation using Watershed with post-processing

We first define a two-dimensional gray-scale image $\mathbf{I}$ as: $D_I \subset \mathbb{Z}^2$. Consider an arbitrary pixel $\forall P \in \mathbf{I}$, which has a gray level $G_P \in [0, N]$, where N stands for the value of the highest gray level of $\mathbf{I}$. After smoothing the original image with the help of a 3×3 Gauss filter, we calculate the gradient image, which acts as the input of Watershed algorithm (Luc & Pierre, 1991). Let N_P denotes the neighborhood of pixel P , and the operation can be expressed as:

$$Grad_p = \sqrt{\sum_{p' \in N_p} (G_{p'} - G_p)^2} \quad (1)$$

Watershed algorithm is dedicated to marking a coarse cell pixel set $\mathbf{W} \subseteq \mathbf{I}$ and the corresponding boundary. In case of over-segmentation resulted from the watershed algorithm, $Grad_p$ would be concentrated on a significant gray level of the gradient image compared with the minimum of cell boundary pixels' gradient. It is worthwhile mentioning that finite element geometric context information, e.g., size of a cell, could be associated with an empirical threshold to separate the relevant parts from the irrelevant parts. It is observed that the size of set $\mathbf{W}$ is larger than what is expected, and further processing is needed to detect and refine the boundary.

Then a method is introduced to connect coarse segmentation with further refinement together. First, a dilation algorithm is employed to ensure set $\mathbf{W}$ cover all parts of the cell. Set $\mathbf{W}$ is updated after dilation. We consider a set $\mathbf{B} \subset \mathbf{W}$ including the pixel which has more than one unmarked pixel and more than one marked pixel in its neighborhood as the boundary of cell. This property of set $\mathbf{B}$ can be described as:

$$N_{P_b} \cap (\mathbf{I} - \mathbf{W}) \neq \emptyset, \forall P_b \in \mathbf{B} \text{ and respectively } N_{P_w} \cap (\mathbf{I} - \mathbf{W}) = \emptyset, \forall P_w \in (\mathbf{W} - \mathbf{B}).$$

where P_b and P_w are points from set $\mathbf{B}$ and $\mathbf{W} - \mathbf{B}$, respectively. After this step, we get a smaller area $\mathbf{W}$ that contains cell and an initial boundary $\mathbf{B}$.

Then adaptive erosion algorithm is adopted to deal with the accurate segmentation no matter what kind of boundary the cell has. All boundary pixels $\forall P_b \in \mathbf{B}$ will move towards the inside part of the cell by certain distance to closest local gradient maximum. The moving direction should be from set $\mathbf{I} - \mathbf{W}$ (outside of cell boundary) to set $\mathbf{W}$ (inside of cell boundary). A 3×3 operator is used to decide the moving direction (Fig. 2), in which boundary pixels will move to the point with the highest gradient. The movements of pixel will generate a "trace" denoted by set T_{P_b} . We expand the width of trace to 3 pixels denoted by $\mathbf{T}'_{P_b}$ and convert the status of pixels covered by the trace from "inside pixel" to "outside pixel". A new set $\mathbf{W}$ can be formed by: $\mathbf{W} - \mathbf{T}'_{P_b}, \forall P_b \in \mathbf{B}$. After all boundary pixels have been moved, a new boundary pixel set $\mathbf{B}$ and a smaller $\mathbf{W}$ can be detected by close operation. Fig. 3 provides a typical example of the proposed adaptive erosion algorithm.

2.2 Microscopy compensation using mean shift filtering

Certain artificial noise and interference cannot be avoided during the image acquisition. Taking our data set as an example, the images contain some white regions which represent noise introduced during the image acquisition. As these regions have stronger boundaries than the real cell boundaries and usually present near the desired boundary details, they have destructive influences on the accuracy of the segmentation. In order to avoid such destructive influence, we process set $\mathbf{W}$ with a more specific method. Considering these parts have different gray levels and properties which are hard to remove from $\mathbf{W}$ with ordinary erosion method, we employ mean shift filtering to compensate the target image and facilitate further image processing.

The mean shift estimation of the gradient of a density function and the associated iterative procedure of mode seeking have been developed by Fukunaga and Hostetler (1975).

Cheng's Paper (1995) attracted attention for wider applications of this algorithm. Mean shift is a tool for finding modes in a set of data samples, manifesting an underlying probability density function (PDF) in feature space.

Given n data vectors $V_i, i = 1, \dots, n$ in the 2-dimensional Euclidean space R^2 . The multivariate kernel density estimate obtained with kernel $K(V_P)$ and window radius h , computed in the point P is defined as:

$$\hat{f}(V_P) = \frac{1}{nh^2} \sum_{i=1}^n K\left(\frac{V_P - V_i}{h}\right) \quad (2)$$

where the kernel $K(V_P)$ is a bounded function with compact satisfying conditions (Zhao et al., 2005).

The use of a differentiable kernel allows defining the estimation of the density gradient as the gradient of the kernel density estimate (2):

$$\widehat{\nabla} f(V_P) = \hat{f}(V_P) \frac{4}{h^2} M(V_P) \quad (3)$$

where

$$M(V_P) \equiv \frac{1}{n} \sum_{P' \in N'_P} [V_{P'} - V_P] = \frac{1}{n} \sum_{P' \in N'_P} V_{P'} - V_P \quad (4)$$

is called the sample mean shift. The region N'_P is a hypersphere of radius h , centered on P , and contains n data points. Since the mean shift vector always points to the direction of the maximum increase in the density, it can define a path leading to a local density maximum. In consequence, mean shift is proposed and widely accepted as a method of image clustering while we use it as an edge preserving filtering method.

A further development of the mean shift procedure was proposed in 1999 by Comaniciu (Comaniciu & Peter, 1999), (Comaniciu & Peter, 2002). The main idea of his approach is to apply the mean shift procedure for the data points in the joint spatial-range domain. We adapt his work as a clustering method and a discontinuity preserving filter to cluster the white noise into one set while preserving the boundary. This procedure can be described as follows:

Mean Shift Filtering Algorithm

For all $P \in I$:

Step 1. Initialize each pixel as a vector $V_P = (x, y, G_P)$, and set the number of iteration: $K = 1$.

Step 2.

$$V_P^{K+1} = \frac{1}{n_K} \sum_{P' \in N'_P} V_{P'}$$

To each pixel, compute new mean shift vector as described in (4):
convergence. In which N'_P is a window set centered on P with spatial radius h_s and gray level radius h_r .

Step 3.

Assign $V_P = (x, y, G_P^K)$ as the result.

To illustrate the effectiveness of the filtering process, a filtering example is presented in Fig. 4 and Fig. 5. The region marked in Fig. 4(a) and (b) is represented in three dimensions in Fig. 5(a) and (b). Raw pixels (Fig. 4(a) and Fig. 5(a)) have been processed with a mean shift filter having $(h_s, h_r) = (6, 30)$. As a result, the filtered data (Fig. 4(b) and Fig. 5(b)) shows a clean and contrasting boundary and quasi-homogeneous regions.

After the mean shift procedure, we can continue with the erosion algorithm. It is defined as follows:

$$\text{For } \forall P_b \in \mathbf{B}, \text{ remove } P_b \text{ from set } \mathbf{W} \text{ if } \exists P' \in \mathbf{N}_{P_b} \text{ and } G_{P'} > G_w$$

assuming the artificial noise and interference pixels' gray level is higher than G_w after mean shift filtering. After checking all the boundary pixels, a more accurate boundary set $\mathbf{B}$ can be detected. To repeat this algorithm till all the white parts have been removed.

After performing the erosion algorithm, we can use the tighter boundary set $\mathbf{B}$ as the input of the detail detection and preservation algorithm which is steered by biological information. In the next section, we are going to discuss the multi-scale detail detection algorithm based on B-spline interpolation.

2.3 Detail confidence calculation using multi-scale information

The set $\mathbf{B}$ derived from Section 2.2 forms a spiky boundary. Any algorithm based on this boundary cannot be a robust one, so this boundary should be further processed before detail detection. Set $\mathbf{B}$ can be considered as a set of scattered data. A lot of work on scattered data interpolation has been made (Franke & Nielson, 1991). Taking the computational complexity into consideration, we choose an algorithm based on B-spline wavelets (Wang & Lee, 1998). We briefly describe the B-spline theory used in Appendix A (Unser et al., 1993).

We start B-spline interpolation with using a B-spline basis to process the boundary set $\mathbf{B} = \{(x_1, y_1), (x_2, y_2) \dots (x_n, y_n)\}$ where the neighbor elements are corresponding to the neighbor pixels in original cell boundary and parameterize the curve:

$$C_0(u) = [x(u), y(u)]^T = c_0(u) * N^l(u) \quad (5)$$

where $N^l(u)$ is the B-spline basis function and $c_0(u)$ is the approximation coefficient vector decided by set $\mathbf{B}$. The details are given in Appendix A.

Then we can get the curve at different resolution level s by convolving the curve with the discrete sampled B-spline of order n at resolution s (Franke & Nielson, 1991):

$$C(u, s) = [x(u, s), y(u, s)]^T = C_0(u) * d_s^n(u) \quad (6)$$

Having finished the scattered data interpolation, we can start to use the previous results $C_0(u)$ and $C(u, s)$ to calculate detail confidence. The ordinary detail detection method uses (7) to calculate all points' curvature $\kappa(u)$ and forms a curvature map (Fig. 6).

$$\kappa(u) = \left| \frac{x_{uu}y_u - y_{uu}x_u}{x_u^2 + y_u^2} \right| \quad (7)$$

where x_u , x_{uu} and y_u , y_{uu} are the first and second derivatives of $x(u)$, $y(u)$ respect to u in (5). Serious false positives cannot be avoided if we use this method. So we implement the detail calculation in a multi-scale fashion to improve robustness. In order to investigate information at different scales, typical curvature maps at four different scales are given in Fig. 6(a)–(d) by (N).

$$\kappa(u, s) = \left| \frac{x_{uu}(s)y_u(s) - y_{uu}(s)x_u(s)}{x_u^2(s) + y_u^2(s)} \right| \quad (8)$$

where $x_u(s)$, $x_{uu}(s)$ and $y_u(s)$, $y_{uu}(s)$ are the first and second derivatives of $x(u, s)$, $y(u, s)$ respect to u in (6) and S indicates the scale level.

After finishing the calculation of multi-scale curvature information, the detail confidence can be calculated on the basis of propagation through scale space. We use the following algorithm to combine multi-scale information and get a final result.

Multi-scale Information Combine Algorithm

Step 1.

For all scale levels from 1 to l_{max} : sort all pixels according to their curvature. A value $R_p^l \in [0, 1]$ indicates pixel P 's importance (curvature rank) at level l .

Step 2.

For scale levels from 2 to l_{max} : if all $P' \in N_p''$ has $R_{p'}^{l-1} < R_p^l$, then set $R_p^l = \frac{1}{2n+1} \sum_{P' \in N_p''} R_{p'}^{l-1}$, where N_p'' is P 's boundary pixel neighborhood of radius n .

Step 3.

For all pixels: let $D_p = R_p^{l_{max}}$.

A boundary pixel importance set **D** which describes the importance of boundary pixels by value $D_p \in [0, 1]$ will be calculated after this step. The value $D_p = 1$ indicates this pixel is the most important detail pixels while $D_p = 0$ indicates the least possibility of being a detail pixel.

Once the boundary pixel confidence set **D** has been calculated, interactive mechanism between low-level contour model and high-level interpretation can be achieved by integrating the cell details information into the proposed snake model. In the next section, we narrate the detail preservation method which employs specially designed energy term in snake model to represent the high-level requirements.

2.4 Detail preservation integrated in snake model

An algorithm should be designed for detecting seed points of snake algorithm. These seed points should be stored in order. For example, we have two cells in the image, so we use two arrays **A**₁ and **A**₂ to store the seed points. In each array, the seed point P_n 's neighbor unit (P_{n+1} and P_{n-1}) should be its closest connected neighbor seed points in the boundary set **B**. Besides, detail points should be sampled at a higher rate to guarantee that all details can be represented and preserved. Then, the greedy snake algorithm is used to get a more accurate boundary while with all wanted details preserved. Energy in this active contour model is represented by three energy terms: E_{snake} , $E_{penalty}$ and E_{detail} . E_{snake} has three components: E_{con} and E_{cuv} are responsible for maintaining continuity between points by controlling segment length and vector curvature and image energy E_{img} is represented as the magnitude of the gradient. Their description can be found in Appendix B.

In order to integrate the detail confidence which represents biologists' demands such as maintaining certain significant cytoarchitecture details while processing target images, we introduce energy term E_{detail} to control the evolution procedure of the active contour. Fig. 7 demonstrates the effect of this term. Fig. 7(a) is the initial boundary given by Section 2.2 which covers very tiny but important cell details. Without this term, the detail parts of initial boundary set $\mathbf{B}$ will be processed in the same way as ordinary parts, which finally yield to the result shown in Fig. 7(b). Considering the importance of the boundary details, we increase the sampling rate of seed points and control the movements of seed points by adding the term E_{detail} according to the combined multi-scale information from Section 2.3. Fig. 7(c) presents the corresponding processing result.

Energy term $E_{penalty}$ is a penalty term which represents a special external constraint to compensate negative effect introduced by outside strong interference. Fig. 8(a) is the initial boundary given by Section 2.2 which has peak interference near the boundary. Fig. 8(b) shows the processing result without the penalty term. The active contour moved to the outside noise point because the outside peak interference possesses stronger attractive force than the real cell boundary. In order to avoid this situation in cell image processing, we introduce $E_{penalty}$ into the ordinary snake model. Any movement to the outside of set $\mathbf{W}$ would be "punished" but not forbidden, so better performance (Fig. 8(c)) can be achieved as what are shown in the pictures.

The total energy of $\forall P'_n \in \mathbf{N}_{p_n}$ is described by (9):

$$E_{P'_n} = E_{snake} + E_{penalty} + E_{detail} \quad (9)$$

where $E_{penalty} = \gamma E_{snake}$ and γ is the penalty function which can be described by (10):

$$\gamma = \begin{cases} -\frac{1}{L} & P'_n \in \mathbf{W} - \mathbf{B} \\ 0 & P'_n \in \mathbf{B} \\ L^2 & P'_n \in \mathbf{I} - \mathbf{W} \end{cases} \quad (10)$$

where L is the distance between P'_n and set $\mathbf{I}$.

$E_{detail} = \mu E_{snake}$ and μ can be described by (11):

$$\mu = \begin{cases} 0 & D_p < 0.95 \\ D_p & D_p \geq 0.95 \end{cases} \quad (11)$$

where the threshold is in charge of controlling detail preservation intensity. Generally speaking, we consider 5% of the boundary points to be detail points, so the threshold is taken as 0.95.

Then the algorithm can be represented as: For all $P_n \in \mathbf{A}_x$, we use (9) to get $P'_n \in \mathbf{N}_{p_n}$ which has the minimum energy in set $\mathbf{N}_{p_n}$. Then we compare E_{P_n} with $E_{P'_n}$ and replace P_n with P'_n if $E_{P_n} > E_{P'_n}$. Then we are able to generate new boundary pixel set $\mathbf{B}$ and cell pixel set $\mathbf{W}$ with the updated seed points set $\mathbf{A}_1, \mathbf{A}_2, \dots, \mathbf{A}_X$.

3 RESULTS

The proposed approach has been validated by applying it to two image series containing 20 images of cell migration and other typical images supplied by biologists. Each image contains two or more cells.

3.1 Research target and image acquisition

Our primary research target is mouse 3T3 fibroblasts. Fig. 9(a) includes two 3T3 cells which were maintained on the microscope stage at 37°C in Dulbecco's modified Eagle's medium (Sigma) supplemented with 10% donor calf serum, 2 mM L-glutamine, 50 $\mu\text{g/ml}$ streptomycin and 50 U/ml penicillin.

The original microscopic images, 640×480 pixels in size and in 256 gray levels, were acquired by a Zeiss IM-35 microscope equipped with a 40X, NA 0.65 Achromat phase objective lens, a stage incubator and a cooled CCD camera (Mintron 12V1E-EX, Santa Clara, CA). Images were taken 15 hours after the cells were plated on the polyacrylamide substrate at a low density while the time interval of taking each image is 2 minutes.

3.2 Results of mean shift filter and microscopy compensation

Fig. 9(b) provides the result of mean shift algorithm having $(h_s, h_r) = (6, 30)$. Compared to Fig. 9(a), Fig. 9(b) offers us an evidence of the effect of applying the mean shift filter. It is clear that the white noises caused by microscopy have been clustered to the same set and have the same gray level which would generate a tighter boundary to facilitate further processing. The selection of (h_s, h_r) is based on the analysis of noise properties which can be considered to be compact in spatial space and patulous in range space. Note that although a careful selection of (h_s, h_r) is helpful, mean shift algorithm is relatively not sensitive to input parameters.

3.3 Comparison between ordinary and multi-scale detail confidence calculation methods

Fig. 10(a)–(c) are the results of ordinary detail detection confidence calculation stated by Marias (2005) while Fig. 10(d)–(f) are the results of the multi-scale method stated in Section 2.3. Blue lines are the input boundaries and red lines are the detected details. Compared to Fig. 10(d), Fig. 10(a) presents several inaccurate detection points because of the fuzzy and low contrast cell boundaries. As to Fig. 10(e), it is easy to notice that Fig. 10(b) also has the same problem with detail detection; furthermore, it has missed one significant detail of the left cell. We notice that the same problem has occurred to Fig. 10(c) because of the utilization of the ordinary detail detection method.

3.4 Full processing results

A series of continuous operational phrase are shown to track the convergence performance of the proposed algorithm. As shown in Fig. 11(a), we can see that the watershed transform will not work in our biological applications because of the low contrast boundaries the cells behave and much environmental noise introduced during the acquisition. After employing post-processing of watersheds, we obtained the result presented by Fig. 11(b). Note that the cell pixel set, **W**, should cover all parts of the interested cell for further refinement.

Fig. 11(c) demonstrates the results after initial segmentation which includes the watershed transform and post-processing. We begin the detail detection with microscopy compensation after which the results are shown in Fig. 11(d). A spiky but more accurate boundary set **B** is generated after this stage. Multi-scale detail detection method is employed to detect cell details showed in Fig. 11(e).

With detail confidence calculation algorithm integrated in snake model accompanied by additional pre or post-processing procedure, we obtain a cell boundary with second degree continuation which can facilitate the following researches. Meanwhile, the parts which could be the most interested details are specified. Our final segmentation result is presented in Fig. 11(f). The procedure of refine segmentation can be iterated for a several times while the number of iteration is decided according to the accuracy demanded of segmentation and detail preservation results. In Fig. 11(g), we illustrate the manual segmentation result. We evaluate our approach by comparing the final segmentation result with manually segmented image. As we can see, the two results are very close except a few minute differences. Quantitative result is presented in Table 1 by measuring the percentage of the overlapping area of automated and manual segmentation. The result is further ameliorated after every processing steps of our approach. More segmentation results from individual sequences are given in Fig. 12, where the visual quality images as subjective evidence characterize representatively the most difficult behaviors with regard to low SNR and fuzzy, irregular, and ruffling cell boundaries during the time-lapse cell migration sequences.

3.5 Sequence processing results and compatible test

We end this section by using our approach to process two sequences from time-lapse microscopy. There are 8 images in sequence 1 and 12 images in sequence 2 and each image in the sequences contains at least two 3T3 cells. Those imposed images are characterized of different types of dynamic cells with spurious branches, arbitrary gaps, low contrast boundaries and low signal-to-noise ratio. The quantitative results are calculated using the method given in Section 3.3 and presented in Table 2. Although our major target is process 3T3 cells from time-lapse microscopy, visual results of other typical images are also given in Fig. 13 where the green is for cell actin staining, using Rabbit anti-actin polyclonal antibody (CHEMICON International Company) and the cells are human breast cancer cell line T47D to prove the compatibility of the proposed method. It also proves the possibility of our scheme's application in other domain.

4 CONCLUSION

This paper introduces a new approach for accurate segmentation of cell migration imaging studies based on the novel detail confidence and detail preservation concept. Our approach can be partitioned into three steps: initial segmentation, microscopy compensation and calculation and integration of detail confidence. The watershed algorithm with post-processing is first deployed to get a coarse boundary and then the mean shift algorithm is used to remove the background noise caused by microscopy interference. After the microscopy compensation, we use the B-spline interpolation followed by the detail confidence calculation procedure to detect and preserve boundary details and apply the improved snake algorithm. A full data processing pipeline diagram is given in Fig. 14. With the high-level information fully explored and utilized, we have demonstrated that our confident shape representation framework can achieve a tight and smooth boundary with most of the boundary details well preserved. The proposed method could impose on time-lapse microscopy images with spurious branches, arbitrary gaps, low contrast boundaries and low signal-to-noise ratio.

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Appendix A

The generic space of polynomial splines of order n is denoted by C_l^n where subscript represents the spacing between the knots $P_i (i = 0, 1, \dots, l)$.

$$C_l^n(u) = \sum_{i=0}^l P_i N_i^n(u) \quad (12)$$

where the continuous B-spline of order n (I. J. Schoenberg, 1969) $N^n(u)$ is denoted by:

$$N^n(u) = \sum_{j=0}^{n+1} \frac{(-1)^j}{n!} \binom{n+1}{j} \left(u + \frac{n+1}{2} - j\right)^n \cdot \mu\left(u + \frac{n+1}{2} - j\right)$$

with $u \in R$ and $\mu(u) = \begin{cases} 0, & u < 0 \\ 1, & u \geq 0 \end{cases}$

(13)

Then the discrete sampled B-spline of order n at resolution m $d_m^n(k)$ which is obtained by directly sampling the n th-order continuous B-spline at the scale m is denoted as:

$$d_m^n(k) = \frac{1}{m} N^n\left(\frac{k}{m}\right) \quad \forall k \in Z \quad (14)$$

Appendix B

The energy term E_{snake} of $\forall P'_n \in \mathbf{N}_{p_n}$:

$$E_{snake} = E_{con} + E_{cuv} - E_{img} \quad (15)$$

where E_{snake} 's components:

$$E_{con} = |P_{n+1} - P'_n| - \frac{1}{m} \sum_{P_x \in A_x} |P_{x+1} - P_x| \quad (16)$$

$$E_{cur} = |P_{n+1} + P_{n-1} - 2P'_n| \quad (17)$$

where m is the number of points in array $\mathbf{A}_x$.

Image energy E_{img} is represented as the magnitude of the gradient described by (11):

$$E_{img} = -\text{Grad}_{P'_n} \quad (18)$$

Figure 1. Block diagram of image segmentation system with feedback. Analysis is applied to raw image to collect information which can be utilized to refine segmentation results.

Figure 2. Example of a searching direction operator. The searching direction parallels the normal direction of the boundary.

Figure 3.

Example of adaptive erosion algorithm. Pixels within the expended trace set T'_{p_b} will be removed from set W.

(a)

(b)

Figure 4. 3T3 cell images: (a) original image; (b) the result of mean shift filtering, boundaries are partially enhanced which will benefit the final segmentation result in the blue rectangle.

Figure 5. The gray-level distribution of the area marked by blue rectangle in Fig. 4. (a) original data. (b) mean shift filtering result using $(h_s, h_r) = (6, 30)$.

Figure 6.

Curvature maps at four different scales, from fine to coarse, respectively. Curvature maps at finer scales supply more information while curvature maps at coarser scale supply less but more accurate information.

Figure 7. Example of boundary detail of 3T3 cell. By adding the term E_{detail} according to the combined multi-scale information, details of 3T3 cell are well preserved.

Figure 8. Example of boundary detail of 3T3 cell with nearby peak interference. This case demonstrates the effect of the energy term $E_{penalty}$ which compensates negative effect introduced by outside strong interference.

Figure 9.

(a) Original image of two 3T3 cells; (b) result image of mean shift filtering. White noises caused by microscopy have been clustered to facilitate further processing.

Figure 10. Comparison of detail detection methods, blue line: input boundary, red line: detected details. Wrongly detected details are marked with red rectangles and lost detail is marked with green rectangle.

(g)

Figure 11.

(a) 3T3 cell image segmented by watershed transform; (b) result of watershed's post-processing; (c) result after initial segmentation; (d) result after microscopy compensation; (e) result of detail detection, blue line: initial boundary, green line: boundary after B-spline reconstruction, red line: details; (f) final segmentation result; (g) manual segmentation result.

Fig 12.
The segmentation results of representatively difficult 3T3 cell images in time-lapse microscopy sequences.

Fig 13. Results of other typical images. (a) & (b) show the result of cell actin staining, using Rabbit anti-actin polyclonal antibody; (c) is human breast cancer cell line T47D.

Figure 14. Block diagram to illustrate the data processing pipeline of the proposed method.

Table 1
Similarity between automated and manual segmentation (presented in the form of overlapping area percentage)

Watershed segmentation		Object-aware snake segmentation			
Fig. 11(a)	Fig. 11(b)	Fig. 11(c)	Fig. 11(d)	Results after first iteration	Results after second iteration (Fig. 11(f))
72.87%	80.36%	89.90%	92.81%	94.88%	95.05%

Table 2

Sequence validation results (presented in the form of overlapping area percentage between automated and manual segmentation)

	1	2	3	4	5	6	7	8	9	10	11	12	average	Std dev
Seq 1	91.64%	88.86%	90.45%	92.28%	90.35%	91.65%	91.96%	91.32%					91.06%	1.096%
Seq 2	91.49%	93.41%	91.95%	92.64%	91.41%	93.21%	92.52%	93.26%	92.05%	91.38%	91.56%	93.88%	92.40%	0.713%