COMPUTATIONAL SCRATCH ASSAY - A NEW FRONTIER FOR IMAGE ANALYSIS: PRELIMINARY STUDY OF MULTI-CELLULAR SEGMENTATION

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ABSTRACT

Quantitative scratch assay is significant in cell motility study for tissue repair, evolution of disease, drug treatment, and cancer metastasis. To overcome challenges in traditional manual operations in scratch assay, computational scratch assay is introduced, where image processing algorithms are exploited for cell motility quantification. In this new research realm, dedicated analysis tools are under-developed, which provides many opportunities for researchers expert on signal processing. This work presents a preliminary study in multi-cellular segmentation, which aims to divide a scratch image into wound area and cell-populated regions. The proposed segmentation algorithm consists of a novel LBP-variant edge detector and a parallel processing pipeline. Experimentation on public scratch image benchmark demonstrates the superiority of the proposed method over prior arts. Particularly, the LBP-variant edge detector is capable of generating a single directional-aware edge map so that multiple edge maps along different orientations can be retrieved from it. Taking our preliminary study on multi-cellular segmentation as an example, it is suggested that with carefully designed image processing algorithms, current scratch assay quantification can be much improved.

Index Terms— Wound healing assay, multi-cellular segmentation, computational scratch assay

1. INTRODUCTION

Scratch assay, or wound healing assay, is a classical technique to study collective cell motility and migration for wound recovery, immune function, disease evolution, and cancer invasion and metastasis [1, 2, 3, 4]. In scratch assay, a confluent monolayer of cells are grown under a specific condition, and an artificial wound is created. With time elapsing, cells migrate to scratch areas and tend to close the wound. It should be noted that cell migration is a dynamic processing comprising spatial and temporal information. To record this recovery process, time-lapse transmitted-light techniques are used to generate a sequence of scratch microscopic images over time. Then cell motility is analyzed and quantified based on the sequence of scratch images in terms of multi-cellular tracking, scratch front-edge tracing, wound area measuring, and recovery rate estimation [5].

Traditionally, wound healing assay is performed by specialists manually. However, the complication nature of scratch images poses several challenges for analysis. First, scratch assay manually is not only time-consuming, but also prone to subjective. For instance, to measure wound areas and cell migration rate, the operator needs to identify wound edge based on multi-cellular's locations on each time-lapse image. However, cells at the scratch edges often grow into the wound areas at different rates for recovery, resulting in an ill-defined cell front when time elapses [6]. It has been reported that analysis inconsistency occurs between different operators [7]. Second, to achieve high-quality analysis, quantitative measurement is performed across multiple replicates of the same cell condition in a statistics manner. To this end, wound healing assay may contain as many as 384 well plates of cells [8]. The massive data makes manual operations inefficient, or even infeasible. Therefore, automated scratch assay which can provide objective and efficient analysis is highly desirable.

Computational scratch assay is a research subject that uses image processing tools to analyze cell migration in wound healing images. Fig. 1 depicts a general scheme of computational scratch assay. The input scratch image can be either color or grayscale depending on imaging modalities. After pre-processing, multi-cellular segmentation which divides an image into wound region and cell-populated regions is performed for subsequent quantitative cell mobility analysis. It should be noted that comparing to the developments of computational histo-pathology and fluorescence cell analysis, computational scratch assay is lag behind, and dedicated analysis tools are either under-developed or prone to adopting general image routine algorithms proposed decades before, leading to unsatisfied analysis results. For instance, in the Broad Bioimage Benchmark Collection (BBBC) multi-cellular segmentation benchmark [9], the three examined tools achieve only about 60% F-measure score for scatter image multi-cellular segmentation. One can refer to [5] for computational scratch assay overview. The under-development of computational scratch assay provides signal processing society lots of opportunities and spaces for contributions.

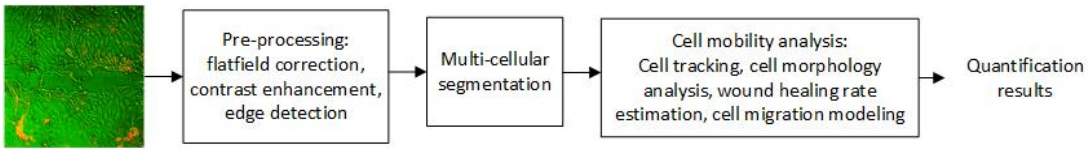

Fig. 1. A general pipeline of computational scratch assay, where most analysis tasks are based on multi-cellular segmentation.

As shown in Fig. 1, multi-cellular segmentation is the basic common computation step for many scratch assay analysis tasks. The accuracy of segmentation is crucial and directly effects precision of further analysis. Despite its significance, multi-cellular segmentation is little addressed by image processing community, with limited studies reported in literature. In this work, we present our very preliminary study on multi-cellular segmentation particularly in scatter images. Different from prior arts that use multiple filters to collect edge information, a novel LBP-variant edge detection operator computes intensity differences along different orientations simultaneously and generates a single orientation-aware edge map. The obtained edge map is then processed by a parallel filtering structure, generating a robust segmentation result. We evaluate the proposed segmentation method using public scratch images, observing that our method achieves much better results compared to prior arts. To clarify, in this study, we say a edge map is direction-aware in the sense that based on a pixel value in the edge map, edge orientation information at that particular pixel can be uniquely retrieved.

The contributions of this work are summarized as follows.

- Computational scratch assay is seldom addressed by signal processing society[1]. This study demonstrates that dedicated-designed algorithms can largely improve the performance of current digital scratch image analysis. We hope this study would encourage more researchers in signal processing society to contribute to this new research frontier.
- We present a very preliminary study on multi-cellular segmentation in scratch images, where a LBP-variant edge detector is introduced in the proposed multi-cellular segmentation method. The obtained single edge map is capable of preserving edge information along various orientations. To the best of our knowledge, this work constitutes the first attempt in literature to translate the LBP-based paradigm to the edge detection scenario.

The rest of this paper is organized as follows. Prior arts in multi-cellular segmentation is reviewed in Section 2. Section 3 presents the proposed segmentation algorithm in detail. Experimental results and discussion are given in Section 4, followed by conclusion in Section 5.

[1]It is hardly to find a work of computational scratch assay published in IEEE Signal processing venues.

2. MULTI-CELLULAR SEGMENTATION REVIEW

Different from classical cell segmentation which aims to segment every single cell, multi-cellular segmentation is essentially a foreground-background segmentation task, where image pixels are labeled by either scratch area or cell-populated regions. The major difficulties in this task are introduced by the high variability in imaging conditions and cells' appearance [9]. The existing multi-cellular segmentation algorithms can be categorized into two groups.

The first category of scratch image segmentation methods is based on edge or texture information in every single image and achieves segmentation in the unsupervised manner. The first freely-available designated tool, namely TScratch [10], is a typical example in this category. It developed edge-detection algorithm based on the discrete curvelet transform in various scales, orientations and positions in an image. Then the obtained curvelet magnitude image is divided into wound area and cell region by thresholding. Later, instead of using the discrete curvelet transform to obtain edge information, Sobel edge detector or Canny method is exploited to generate image edge maps [11, 12, 6]. Alternatively, in Topman's method [13], rather than edge information, texture knowledge, in terms of standard deviation of pixel intensities over a square window, is collected to distinguish wound area and cell-populated regions in scratch images.

The other group adopts supervised learning for wound area segmentation. MultiCellSeg [14] is a dedicated software of multi-cellular segmentation for cell motility. After partitioning an image into small patches, each patch is classified by a linear support vector machine (SVM) with confidence score. Then the score map is segmented by an automatically-selected threshold. Later, a method that augments level set segmentation with a SVM is proposed for scratch assay [15].

In sum, compared with the supervised learning methods whose performance heavily depends on training, the edge-detection based methods are much fast without sacrificing segmentation accuracy [9]. However, we noticed that in prior edge-detection based methods, to obtain accurate segmentation, edge-detection filters in various directions were used to generate multiple edge maps [10, 11, 12, 6]. The resulted multiple maps pose difficulties on data fusion for subsequent analysis. Hence, in this paper, we introduce a multi-cellular segmentation algorithm that uses one edge detection operation to generate a single direction-aware edge map.

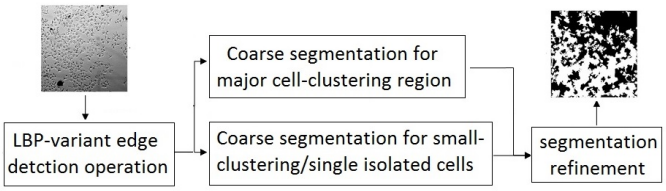

Fig. 2. Block diagram of the proposed multi-cellular segmentation method for wound microscopic images.

3. PROPOSED SEGMENTATION ALGORITHM

Briefly, the proposed multi-cellular segmentation method follows the edge-detection based paradigm for wound healing assay segmentation, where thresholds are used to generate binary segmentation over edge maps. The block diagram of the proposed method is depicted in Fig.2. Given a query wound microscopic image, a single direction-aware edge map is generated using the proposed LBP-variant edge detection operator. Then a parallel coarse segmentation pipeline consisting of two processing paths is applied to the resulting edge map. The finally multi-cellular segmentation is obtained by fusing the two coarse segmentation together.

3.1. Direction-Aware Edge Detection Operation

Generally, two steps are needed to generate edge maps in prior works. First, an image is convolved with an image differential operator (for example, Roberts operator and Sobel operator), obtaining information on intensity differences at each pixel. Then compared to a threshold which can be predetermined or adaptively generated, intensity difference which is larger than the threshold is believed corresponding to an edge. It should be noted that since image edges may be along any directions, edge detection operators in various orientations are needed. In addition, to obtain a single edge image, edge maps associated with different directions need to combine together, where the knowledge of edges' orientations may be lost.

Different from the traditional edge detection paradigm, the proposed edge detection algorithm follows the LBP paradigm [17] and achieves intensity differentiation and thresholding by one operation, generating a single direction-aware edge map. That is, given an edge map generated by the proposed algorithm, edges associated with certain orientations can be retrieved. In specific, given a query scratch image, let image intensities at a pixel $c = (x, y)$ and its P neighborhood pixels be I_c and I_p^n, respectively. To generate a single directional-aware edge map, absolute values of intensity differences between I_c and I_p^ns are computed, forming an intensity difference vector. Then each element of the vector is compared to a predetermined threshold θ. If the intensity difference is larger than θ, the corresponding element of the vector is replaced by 1; otherwise, 0. Finally, the obtained binary code is converted to a decimal integer, represented by e_c, in the edge map. The proposed edge detection operator can be summarized by Eqn. (1) as follows.

$$e_c = \sum_{p=0}^{P-1} s(|I_c - I_p^n| - \theta) \times 2^p, \tag{1}$$

where $s(z) = 1$ for $z \geq 0$; otherwise, $s(z) = 0$. From Eqn. (1), only intensity difference larger than a threshold is considered as an edge and contributes to the value e_c in an edge map.

It is noteworthy that the obtained edge map is said direction-aware in the sense that edge orientations are explicitly indicated by 1s in the binary representation of e_c. That is, based on an e_c, information of edge orientation at pixel c can be uniquely determined. Fig. 3 demonstrates an example of the proposed edge detection operation on a scratch image patch, where edges in different directions in (c)-(f) can be retrieved from the single edge map in (b).

3.2. Parallel Coarse Segmentation Based on Edge Map

With the edge map, we precede to multi-cellular segmentation. Note that compared to classical cell segmentation, multi-cellular segmentation does not need to segment every single cell independently; instead, areas populated by cells should be identified as non-wound regions, even though these regions may not be completely covered by cells. To this end, a two-path parallel filtering structure is exploited in the proposed method, where each path is composed by smooth filtering and thresholding. Specifically, for major cell-clustering region segmentation, a median filter with a large window is applied to the edge map in one path. Consequently, blank areas in cell-populated regions is diminished, eventually preventing over-segmenting non-wound regions. However, a median filter in large window size may lead to segmentation failure for areas taken by small/isolated cell clusters. Such failures are more observed along wound boundary when cells are closing the scratch. Hence, to address this issue, in the other segmentation path, a Gaussian filter with a small window is used to preserve small isolated cell clusters in the edge map. However, the drawback of using a filter in a small window size is over-segmenting large multi-cellular areas.

To obtain a robust multi-cellular segmentation in scratch images, the proposed method combines the two preliminary coarse segmentation generating by the parallel structure using an OR bit operation. That is, a pixel is labeled as a wound pixel if it locates in wound regions in both segmentation obtained from the parallel processing paths.

4. EXPERIMENTAL RESULTS

In this work, the proposed method is evaluated using publicly-accessible scatter images and compared with three designated

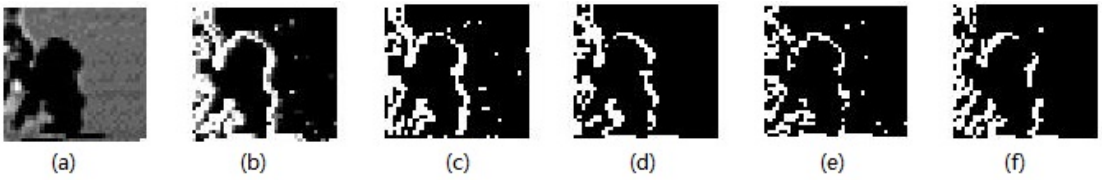

Fig. 3. (a)Scratch image patch; (b) edge map obtained by the proposed detector in Eqn. (1); (c)-(f) edges along different orientations (vertical, 135 degree oblique, horizontal, and 45 degree oblique) retrieved from (b).

Table 1. Evaluation of multi-cellular segmentation algorithms on scatter images

Algorithm	Mean F-measure	Median F-measure
Tscratch [10]	0.514±0.164	0.536
MultiCellSeg [14]	0.611±0.107	0.587
Topman [13]	0.647±0.086	0.616
Proposed	**0.858**±0.042	**0.861**

algorithms, which are TScratch [10], multiCellSeg [14], and Topman's method [13].

Testing Data: Query grayscale scatter images are from the BBBC multi-cellular segmentation benchmark. This dataset contains 6 differential interference contrast images of Madin-Darby Canine Kidney epithelial cells acquired during a multi-well scatter assay [9]. Since manual segmentation is provided as ground truth for 5 images, with one groundtruth missing, only the 5 images with groundthuth are used as testing data.

Experimental Design: Each scratch images is segmented using the proposed method. Following the BBBC scratch array benchmark [9], agreement between segmentation results and ground truth is quantified by F-measure score. F-measure is defined as the harmonic mean of segmentation recall and segmentation precision of wound areas, which is computed by $\frac{2precision \cdot recall}{precison+recall}$. A high F-measure score indicates a better segmentation. In this preliminary study, we set $\theta = 25$, and the window size for the median filter and the Gaussian filter are 25-by-25 and 5-by-5, respectively[2]. We compare the proposed method with prior arts (TScratch [10], multiCellSeg [14], and Topman's method [13]), whose segmentation results are provided by the BBBC benchmark.

Results and Discussion: Statistics of F-measure scores for multi-cellular segmentation evaluation are presented in Table 1. Compared to the three algorithms, the proposed method achieves much higher F-measure scores over the scatter image set. For visualization, an example of multi-cellular segmentation is presented in Fig. 4. Compared to the groundtruth, our segmentation result has some noise (i.e. small white areas). We believe these segmentation errors can be removed by post-processing, for instance, morphology operations. We will discuss the potential improvement in our future study.

[2]The discussion on parameter settings on segmentation performance will be presented in our future works.

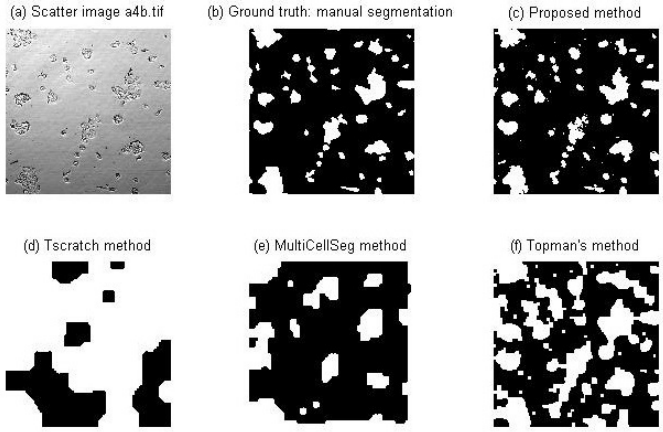

Fig. 4. Example of multi-cellular segmentation in a scatter image obtained using proposed method, TScratch [10], multiCellSeg [14], and Topman's method [13].

5. CONCLUSION

Computational scratch assay is a new research realm, where many analysis tools are under-developed and highly desirable. This paper took multi-cellular segmentation in a scratch image as an example, showing that with carefully designed algorithms, the current scratch assay quantification can be greatly improved. Specifically, based on a single directional-aware edge map obtained by a novel edge detector, a parallel processing pipeline was used to segment both cell-populated areas and small cell-cluster regions. In particular, the proposed detector adopted the LBP paradigm, and the edge orientation information was summarized using a binary code. Consequently, given a resulting edge map, various edge maps in different directions could be retrieved accordingly. Compared to prior arts, the proposed method achieved much more accurate segmentation in terms of F-measure over a public scatter image set. We hoped that this preliminary study would encourage image processing experts to contribute to this research frontier, fostering computational scratch assay research.

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