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PHASE CONTRAST CELL SEGMENTATION USING MACHINE LEARNING APPROACH

Ehab Essa[1], Xianghua Xie[1], Rachel J Errington[2], and Nick White[3]

[1]Department of Computer Science, Swansea University, UK, csehab,x.xie@swansea.ac.uk
[2]Institute of Cancer & Genetics, School of Medicine, Cardiff University, UK, erringtonrj@cf.ac.uk
[3]School of Optometry and Vision Sciences, Cardiff University, UK, whiten@cardiff.ac.uk

SUMMARY

In this paper, we present a machine learning approach based on random forest (RF) for automatic segmentation of living cells in phase contrast images. The proposed method is performed by a multi-stage classification working on both low and high level of the image. Pixel-wise classification is first performed to obtain a probability map of dark and bright cell regions. K-means clustering is then used to group pixels into candidate cell regions. Finally, another RF is called to verify the candidate cell regions. The experimental results show promising performance of the proposed method.

Key words: *cell segmentation, random forest, phase contrast images*

1 INTRODUCTION

Phase contrast microscopy is an optical based technique that converts difference in phase of object light waves into change in intensity which can be displayed as variations in the image. Phase contrast microscopy produces high contract images compared to the bright field microscopy of transparent specimens such as living cells directly without need to be killed, fixed, and stained, so that it is used to monitor cell proliferation in natural and examine the drug effect.

Cell segmentation is an important task in order to analyze cells behavior and track its movement across time-lapse images. Manually segmenting of cells is a time-consuming, laborious process, that can suffer from high inter- and intra-operator variability, specially in the presence of large volume of data captured across time, where each image may contain hundreds of cells. Automatic cell segmentation is still a challenge despite the existence of many methods, due to low contrast between cell and background, inconsistency between the cell structure itself, and the image artifacts such as halo effect.

Many cell detection and segmentation methods in phase contrast images have been introduced based on one or more approaches e.g. thresholding and morphologic operations [21, 4, 8, 22], deformable model [9, 12, 2, 19, 1], watershed [10, 11, 7, 15], graph based model [18, 16, 13] and machine learning [10, 17, 3, 22, 20, 14, 15, 23].

Machine learning methods can be categorized to supervised [10, 17, 3, 22], semi-supervised [20, 15], and unsupervised or clustering methods [14, 23] depending on the mechanism of the learning system used. Some of these methods [17, 3, 14] have been used in cell detection based on initially select a set of candidate points or small regions refereing to the cell location then prune the less likely candidates using leaning-based method. However, these methods are not able to delineate the cell region. He *et al.* [10] proposed to use SVM classifier with wavelet features to highlight cell region and seeded watershed method to separate the cell from the background. The seeds extracted by another AdaBoost classifier. In [23], superpixel clustering is used to segment cells based on learning the cell boundary probability by a random forest classifier. However, these methods may largely over or under estimate the cell region.

Yin *et al.* [22] proposed an artifact-free phase contrast image restoration method by represent the problem as a regularized quadratic cost function so that the cell can be segmented by simple thresholding. A SVM classifier used to identify cell from non-cell. However, this method is not able to

segment bright cells e.g., mitotic cells. Su [20] extends the previous method to segment the bright cell by proposed different restoration method based on the dictionary representation of diffraction patterns. However user interaction is required to define some seeds for a semi-supervised method to correctly classify cells.

In this work, we propose a multi-stage random forest (RF) classifier method to detect and segment cells in microscopy phase contrast images. The first RF classifier is used as a low-level image segmentation to generate a probability map of cell regions. The second RF classifier differentiates the cells from the background noise and returns delineated cells region.

2 PROPOSED METHOD AND RESULTS

Briefly, the proposed method consists of three steps. First, pixel-wise classification is performed using RF to generate a probability map of dark and bright cell regions. Second, K-means clustering is used to group pixels into candidate cell regions. Finally, another RF is proposed to verify the cell identity from the background.

RF [5] is an ensemble classifier from a set of decision trees. RF injects the randomness not only by training each tree on different training sets using a bootstrap sampling but also with a random set of features that is drawn at each node to determine the best tree splitting. In the first stage, we classify image pixels into four categories, i.e. dark cell, bright (mitotic) cell, halo effect, and background. We train RF on two kinds of features, the largest eigenvalue of hessian matrix and the histogram of the pre-conditional features [22], extracted from two sub-windows of size 4, and 8 respectively. The output is treated as a probability of the dark and bright cell location.

A direct segmentation using the binary output of the first RF classifier is prune to mis-segmentation, particularly when cells form clusters. Instead, we carry out a connected component analysis through spatial clustering and morphological process. K-means is an unsupervised clustering in which each pixel can only join one cluster. This achieved by defining a centroid at the initial center of each cluster and assigning each sample in the data set to the nearest centroid by measuring the distance between them, then update the centroid in an iterative manner. We use the k-means clustering to find the peak center of the dark and bright cells. The number of classes is 3. We automatically select the output class corresponds to the cells centers by observing the clustering set that maximizes the probability map computed from the first stage.

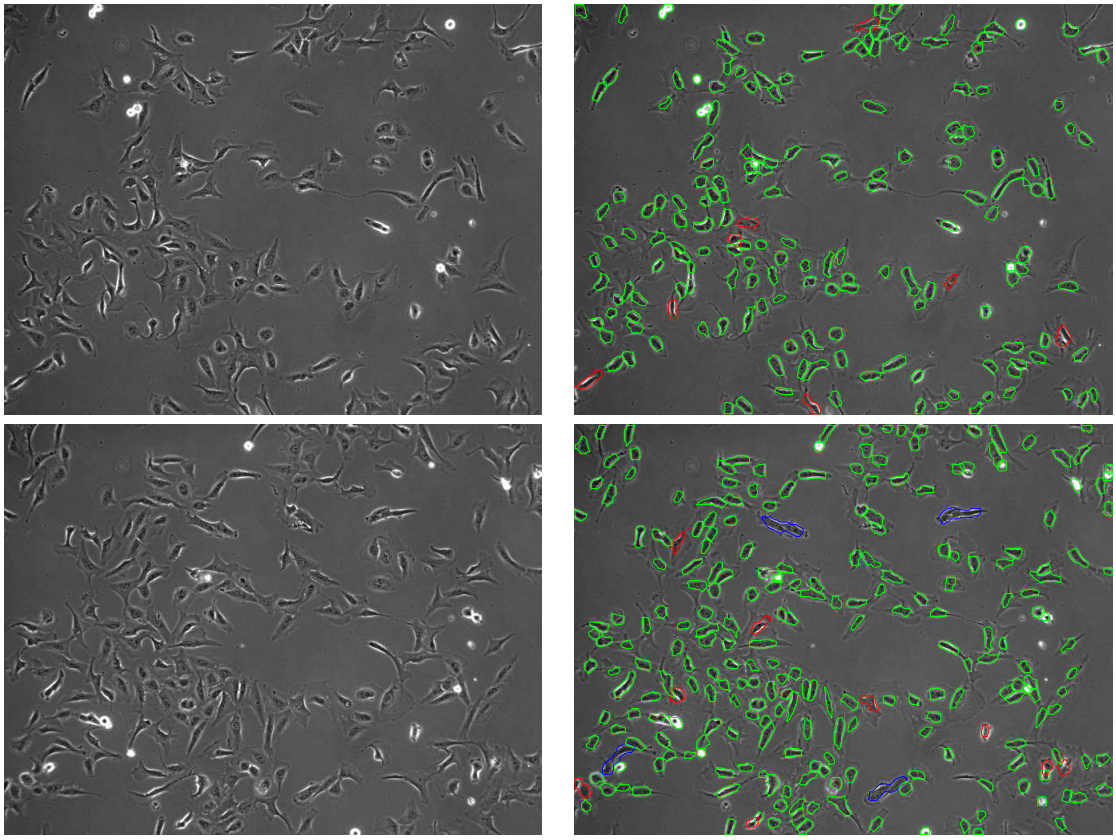
Cell dilation process is then performed to extend the cell region beyond its center. This carried out by converting the probability map into a binary mask and retrieving the region within certain pixel distance. The dilatation process has an advantage that we can easily know if the candidate cells centers are touching each other as they might be a broken cell center or different cells touching. Thus, we create a set of all candidate cell regions, including combine the touching cells region into one set.

In the final stage, we validate the cell identity by using another RF classifier. We classify the initial candidate cell regions into three categories, i.e. single cell, touched cells and background. Histograms of oriented gradients (HOG) [6], and histogram of image intensity are extracted as features from each candidate cell region.

We test the proposed method on phase contrast images of U2-OS human osteosarcoma cells in control conditions. The time-lapse sequence contains 97 images. The training set includes 10 images (2 images to train the first classifier, and 8 images to train the second classifier). Figure 1 shows the final segmentation results. The initial cell region classified as single cell by the second classifier is highlighted by green color. The blue and red color refer to the touching cell and the combined initial cell regions classified as single cell respectively by the second classifier.

3 CONCLUSION

We present a machine learning method to detect and segment the living cells in phase contrast images. Multi-stage RF classifier is proposed to produce a bottom-up cell segmentation. The proposed method shows a promising result despite the segmentation challenges of low contrast and weak edges.



(a) (b)

Figure 1: Cell segmentation results. (a) original image. (b) result image.

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