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Automated Human Embryonic Stem Cell Detection

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***Abstract*— This paper proposes an automated detection method with simple algorithm for detecting human embryonic stem cell (hESC) regions in phase contrast images. The algorithm uses both the spatial information as well as the intensity distribution for cell region detection. The method is modeled as a mixture of two Gaussians; hESC and substrate regions. The paper validates the method with various videos acquired under different microscope objectives.**

Keywords-human embryonic stem cell (hESC); substrate; mixture of Gaussians

I. INTRODUCTION

Human embryonic stem cells (hESCs) are important in the development of regenerative medicine for diseases such as Parkinson's disease, diabetes, etc. The hESCs are pluripotent cells that can differentiate into any cell type. They can prevent embryotoxic chemical, which is a disease causing agent, from releasing into the environment [5] [6]. Therefore, hESCs also have applications in preventive medicine. The detection of hESC regions in phase contrast images is essential for hESCs research. Accurate and fast hESCs region detection can boost the throughput for analyzing hESCs properties and behaviors.

II. RELATED WORK AND CONTRIBUTION

K-means and mixture of Gaussians by Expectation-Maximization (EM) algorithm are widely used techniques for image segmentation. K-means segmentation by Tatiraju et al. [9] considers each pixel intensity values as an individual observation. It partition these observations into k clusters in which each observation belongs to the cluster with the nearest mean intensity value [3] [10]. The method does not consider the intensity distribution of its clusters. On the contrary, the mixture of Gaussians segmentation method by EM (MGEM) algorithm proposed by Farnoosh et al. [7] heavily depends on the intensity distribution models to group the image data. The MGEM method assumes the image's intensity distribution can be represented by multiple Gaussians [4] [8] [9]. However, it does not take into account the neighborhood information. As the result, segmented regions obtained by these algorithms lack connectivity with pixels within their neighborhoods. Their lack of connectivity with pixels within their neighborhoods is due to the following challenges: (1) incomplete halo surrounding the cell body; (2) cell body intensity values are similar to the substrate intensity values. Our proposed method is intended to solve these problems by using spatial information as well as the intensity distribution of the image data. We evolve the cell regions based on the spatial information until the optimal intensity distributions of background and foreground (hESCs) regions are obtained. The proposed method is simple, fast and automated.

III. TECHNICAL APPROACH

A. Mixture of Two Gaussians

hESCs are pipette into the petric dish with a layer of substrate. The substrate becomes the background after the hESCs are placed on its surface. Therefore, we model the hESC image with two regions of interest: foreground and background regions. The intensity distributions of those regions are represented as a mixture of two Gaussians with different mean and variance.

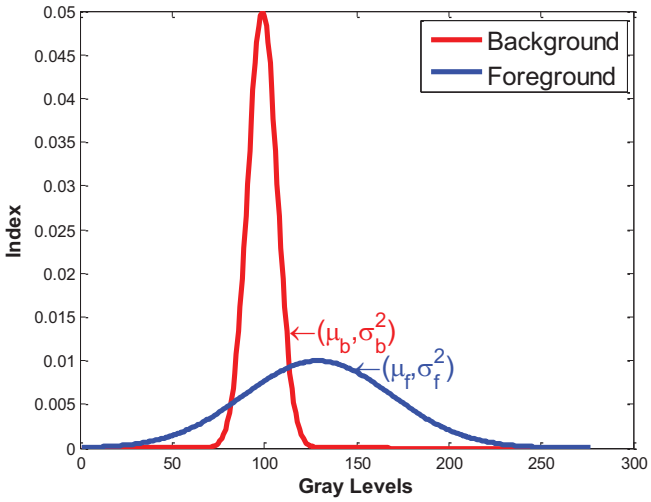

Figure 1: Intensity distribution of foreground and background.

We consider that the foreground is the cell region with mean μ_f and variance σ_f^2, and the background is the substrate region with mean μ_b and variance σ_b^2. Therefore, our problem becomes maximizing the absolute difference of two signal-to-noise ratios (SNRs); the absolute difference of the foreground SNR and background SNR. Equation (4) shows the metric that is used to determine how much the cell

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region data are different from the data of the substrate region. The SNRs of the foreground and background datasets are calculated by the following equations [2]:

$$SNR_f = \frac{\mu_f}{\sigma_f^2} \quad (1)$$

$$SNR_b = \frac{\mu_b}{\sigma_b^2} \quad (2)$$

where SNR_f and SNR_b are the SNRs for the foreground and background datasets respectively.

The metric M is formulated as such:

$$M = \left|SNR_f - SNR_b\right| \quad (3)$$

Substituting equation (1) and (2) into equation (3), we get the following.

$$M = \frac{\left|\sigma_b^2\mu_f - \sigma_f^2\mu_b\right|}{\sigma_b^2\sigma_f^2} \quad (4)$$

Therefore, our solution becomes finding the M_{opt} as described below:

$$M_{opt} = \arg\max_{\mu_f,\sigma_f^2,\mu_b,\sigma_b^2} M(\mu_f, \sigma_f^2, \mu_b, \sigma_b^2) \quad (5)$$

B. Spatial Information and Intensity Distribution

The hESC region, F, is a high intensity variation region while the substrate region, B, is a low intensity variation region. As the result, we exploit the gradients of the image to segment out the cell region from the substrate region. The following equations show how we exploit the gradients of the image:

$$I = F \cup B \quad (6)$$

$$G = \left(\frac{dI}{dx}\right)^2 + \left(\frac{dI}{dy}\right)^2 \quad (7)$$

$$I_G = \log\left(\frac{(-1+e^1)\times G}{\max(G)} + 1\right) \times 255 \quad (8)$$

where G is the squared gradient magnitude of image, I. $\frac{dI}{dx}$ and $\frac{dI}{dy}$ are gradients of image, I, in the x and y direction. I_G is the spatial information result after equation (8), which further emphasizes the difference between cell and substrate region. Equation (8) normalizes G as well as enhances its intensity distribution to become a more visible bimodal distribution.

The proposed algorithm also uses an average filter on I_G at each iteration to evolve the cell regions. It is able to group the cell region pixels together based on local information; the size of the average filter dictates how fast the cell region is evolved. The method updates I_G and evolves the cell region until M is maximized.

Equation (4) is calculated based on the mean and variance of the intensity distributions of cell and substrate data. The cell region , F, and substrate region, B, are updated by thresholding I_G with OTSU's method at each iteration. The intensity distribution's mean and variance of the cell region and substrate region data are also updated at each iteration by the following equations:

$$\mu_f = \frac{\sum_{f\in F} f}{N_f} \quad (9)$$

$$\mu_b = \frac{\sum_{b\in B} b}{N_b} \quad (10)$$

$$\sigma_f^2 = \frac{\sum_{f\in F}(f-\mu_f)^2}{N_f} \quad (11)$$

$$\sigma_b^2 = \frac{\sum_{b\in B}(b-\mu_b)^2}{N_b} \quad (12)$$

Where N_f and N_b are total numbers of foreground and background pixels in the image, f and b are the intensity value in the corresponding foreground and background.

C. Algorithm

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Input:
I: hESC phase contrast image.
Output:
F:the hESC region (foreground).
B:the substrate region (background).

Procedure Cell_Region_Detection(I);
{
Set $M_0 = 0$;
Calculate G and $I_G$with equation (7) & (8).
Spatial grouping by applying an average filter on I_G.
Determine F_1 and B_1 regions by apply OTSU on I_G.
Calculate μ_f, μ_b,σ_f^2 and σ_b^2 with equation (9)-(12)
Calculate M_1 with equation (4).
While($M_i > M_{i-1}$){
    I_G:= spatial grouping by applying an average filter on I_G.
    Determine F_i and B_i regions by applying OTSU thresholding on I_G.
    Update μ_f, μ_b,σ_f^2 and σ_b^2 with equation (9)-12).
    Update M_i with equation (4).
}
F:=F_{i-1};
B:=B_{i-1};
};

==========

IV. Experimental Results

A. Data

All time lapse videos are obtained with BioStation IM [1]. The frames in the video are phase contrast images with 600 x 800 resolutions. The videos are acquired under three different objectives: 10x, 20x and 40x .

B. Parameters

Each video with different objective has a different default size of neighborhood for spatial grouping. The default sizes are determined by observing the ROC plots with various window sizes for each objective. Figure (2) shows that the optimal neighborhood sizes for 10x, 20x and 40x are 5 x 5, 7 x 7 and 11 x 11 windows respectively. The selection criteria of the neighborhood sizes are based on finding a window in which its ROC plot yields high true positive rate while keeping the false positive rate low.

C. Results/Disccusion

The proposed method was tested with three videos that were acquired with 10x, 20x and 40x objectives. Figures (3-5) show the intermediate and final results of the proposed method on those image data. Figure (3b), (4b) and (5b) are

the spatial information when M_{opt} is reached for their respective data. Figure (3d), (4d) and (5d) are the plots of foreground and background's intensity distributions at M_{opt}. The three sets of foreground and background's intensity distributions match our model shown in Figure (1). The foreground distribution of Figure (4d) shows a high probability at its tail. The high probability at its tail is due to the strong presence of halo information in the image. Figure (5) shows the results of a noisy image with the proposed method. The roughness on the foreground and background's distribution curves is because of the noise in the image data.

In this paper, we compare the proposed method with K-means and mixture of Gaussians segmentation methods. Figures (7-8) show the results of the K-means and MGEM segmentation. The results of both methods show the lack of connectivity within their neighborhoods. K-means clusters the image data based only on the nearest mean while MGEM method groups the data solely on the modeled intensity distribution. Consequently, both methods were not able to detect the entire cell regions. Instead, they have detected fragments of the actual cell regions. Their performance is further worsened by the presence of noise as shown in Figure (7c) and (8c). The proposed method as shown in Figure (9) solves the lack of connectivity problem. More importantly, the proposed method's performance is still robust on the noisy image. However, pre-filtering can greatly improve its performance shown in Figure (6) and (10d).

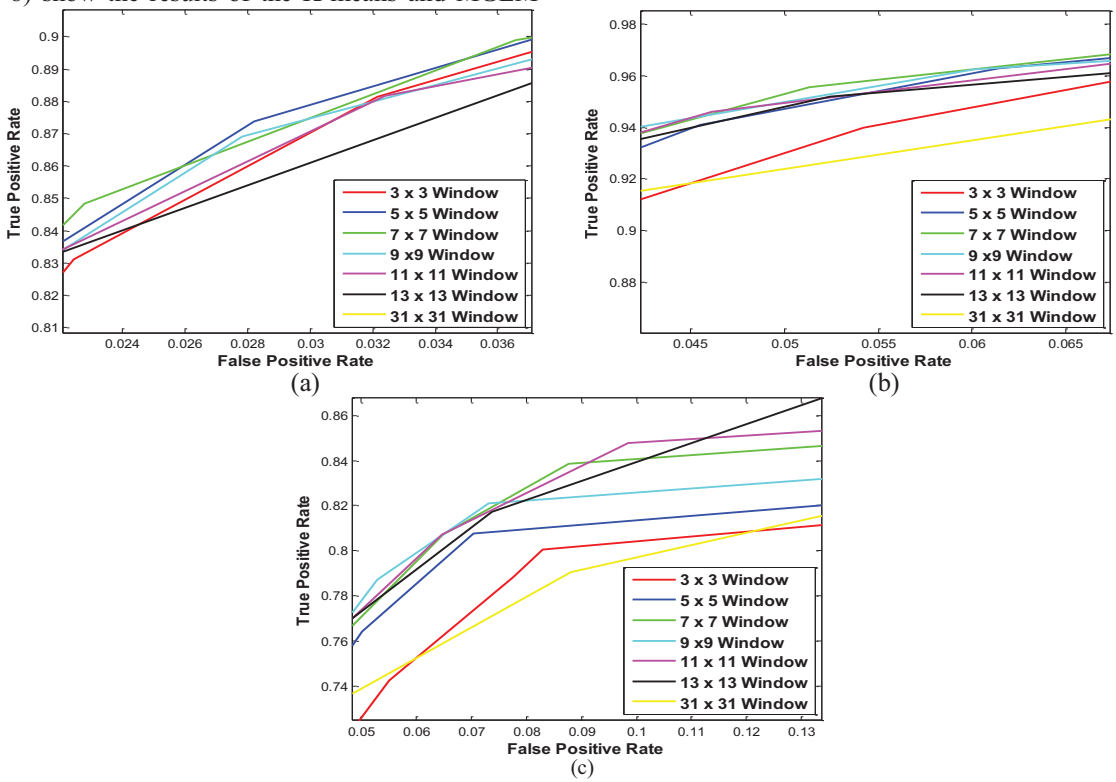
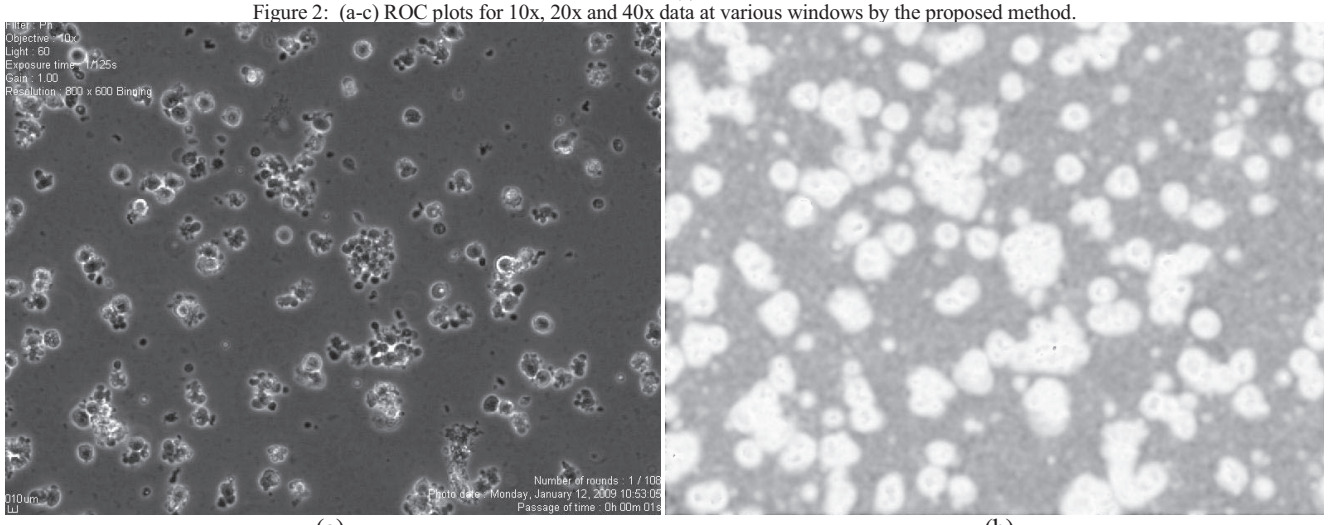
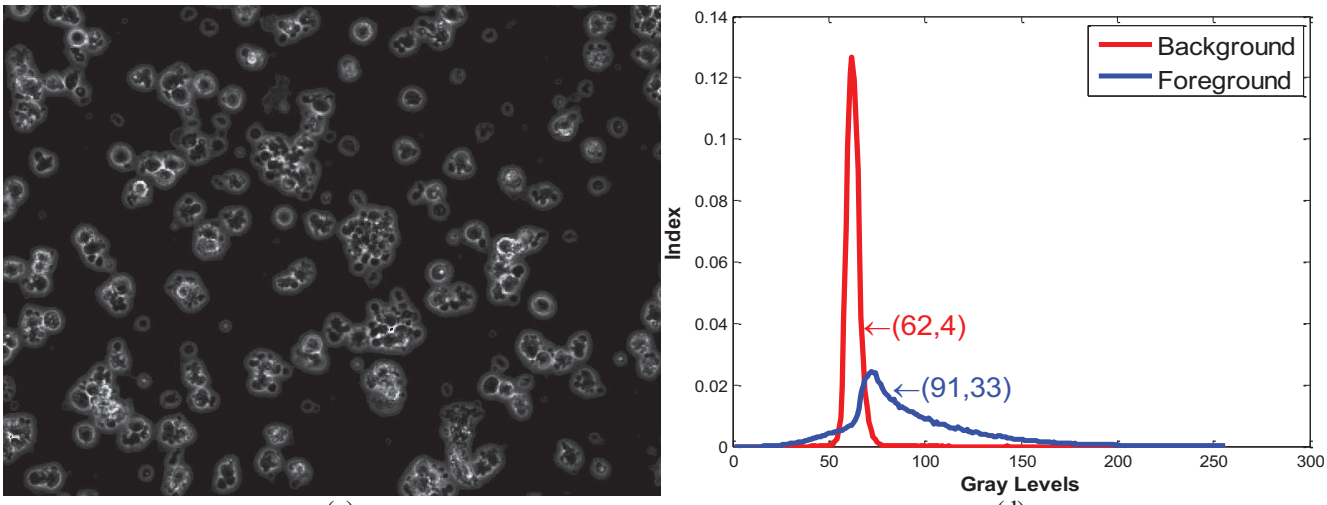


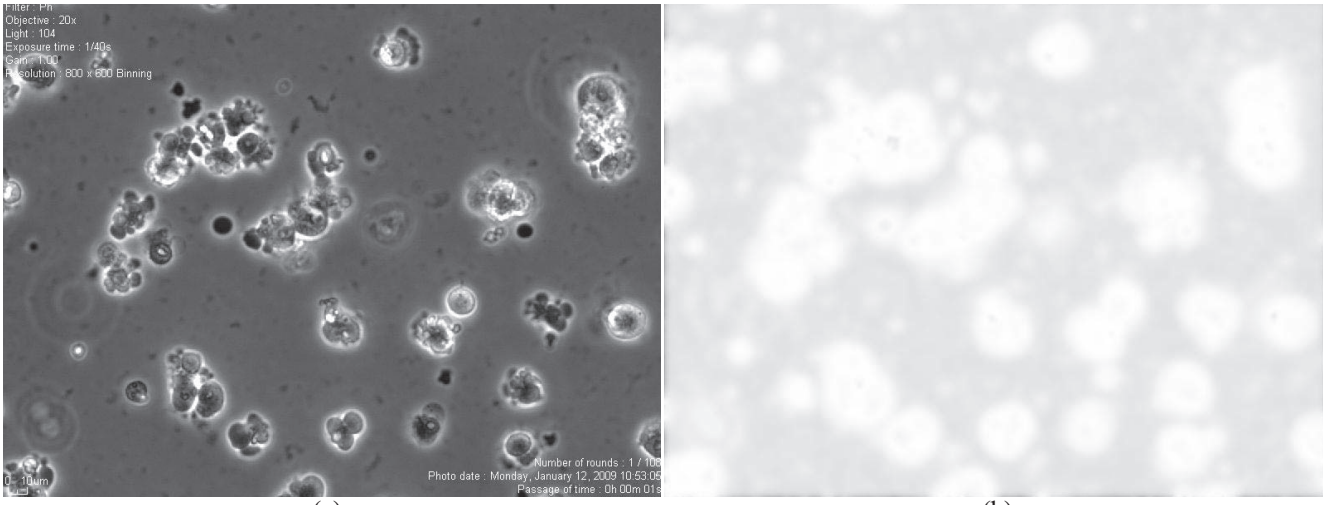
Figure 2: (a-c) ROC plots for 10x, 20x and 40x data at various windows by the proposed method.



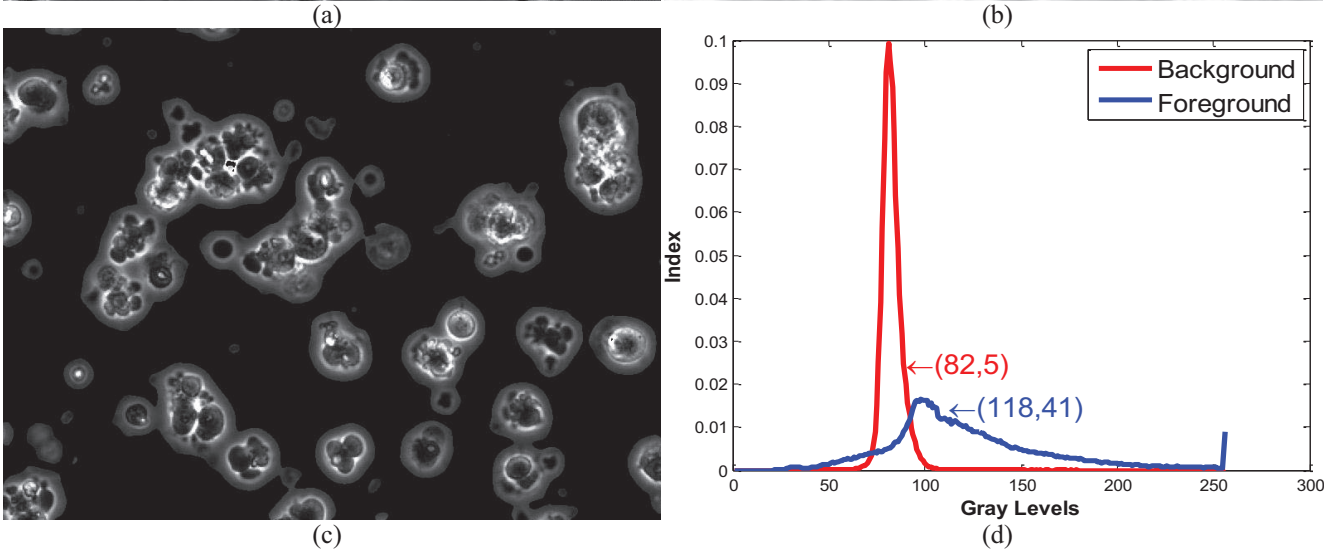


(c) (d)

Figure 3: (a) The original 10x image; (b) spatial information at M_{opt}; (c) detected cell regions; (d) histgram of the foreground and background .



(a) (b)



(c) (d)

Figure 4: (a) The original 20x image; (b) spatial information at M_{opt};(c) detected cell regions;
(d) histgram of the foreground and background.

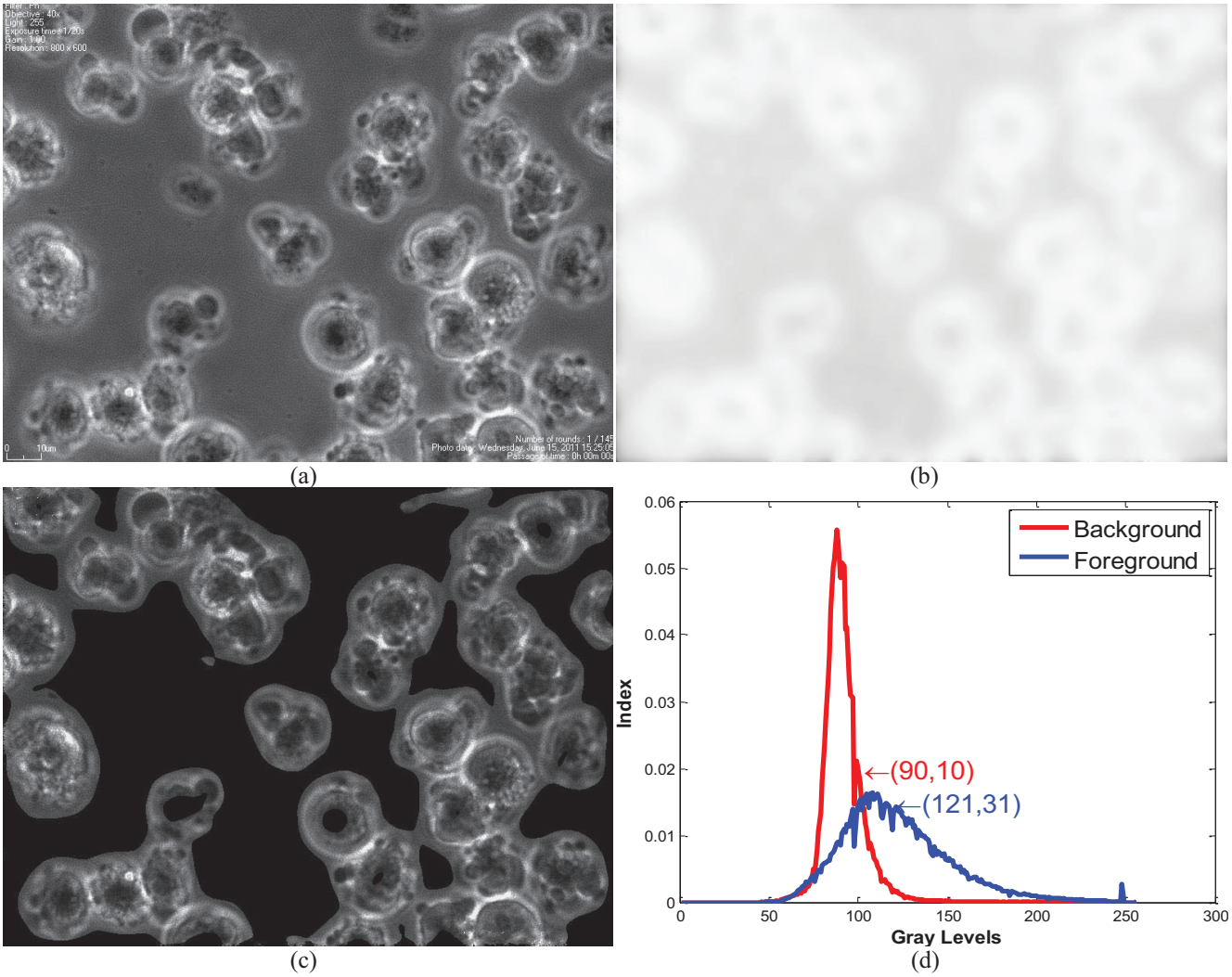
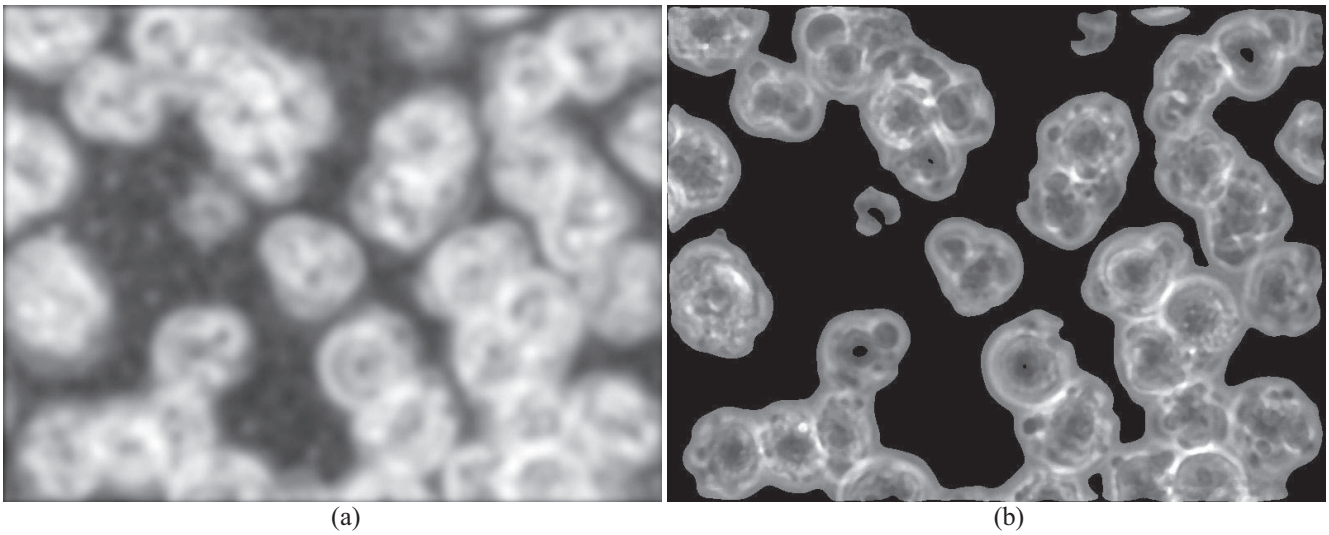
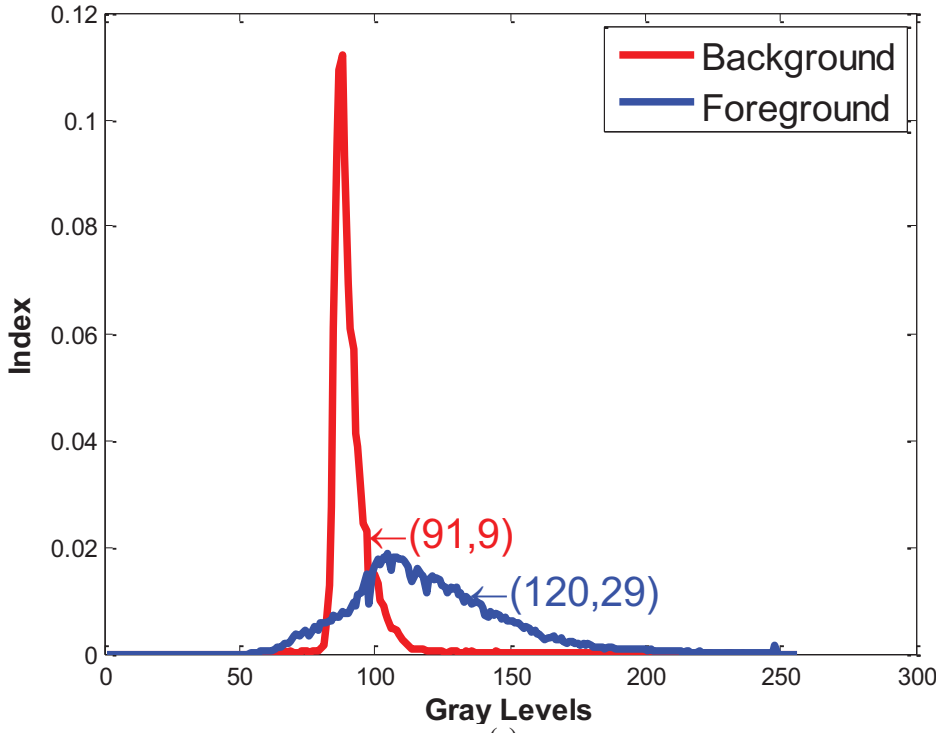


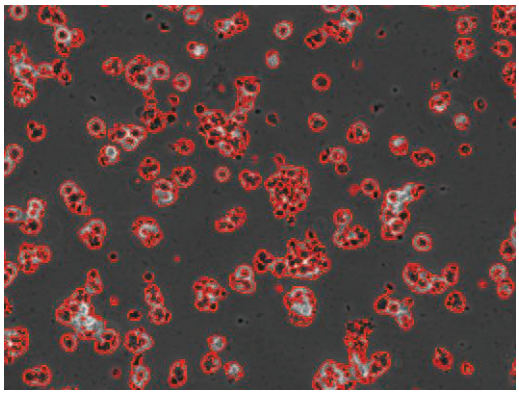
Figure 5: (a) The original 40x image; (b) spatial information at M_{opt}; (c) detected cell regions; (d) histgram of the foreground and background data.



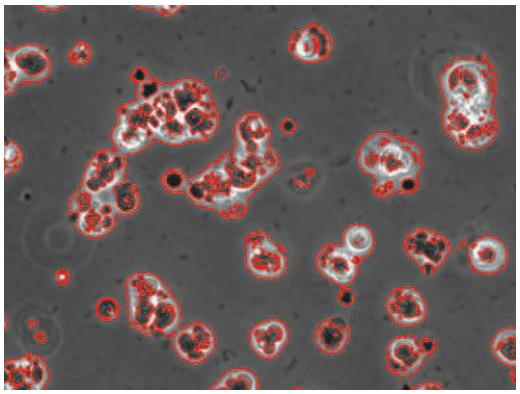


(c)

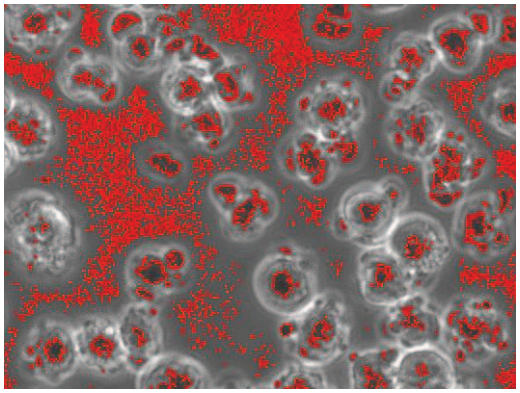
Figure 6: (a) Spatial information at M_{opt}; (b) detected cell regions;
(c) foreground and background histogram of the 40x data after the 5x5 median filter.



(a)

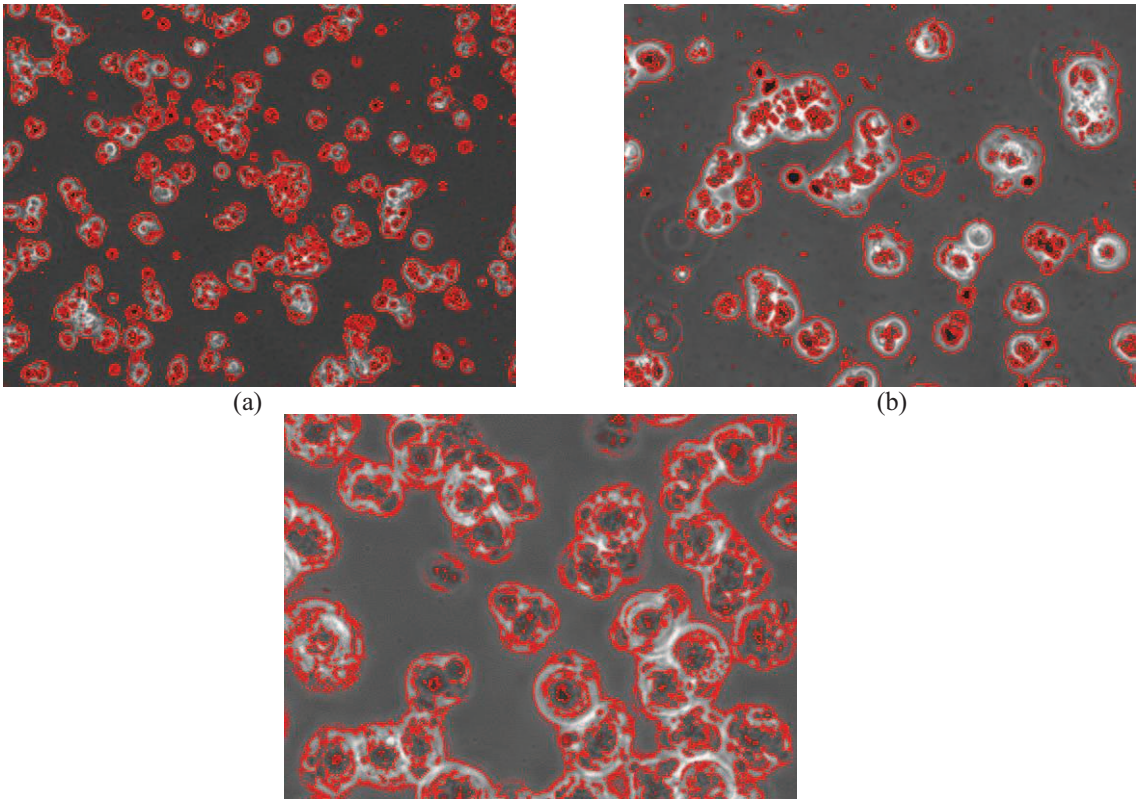


(b)



(c)

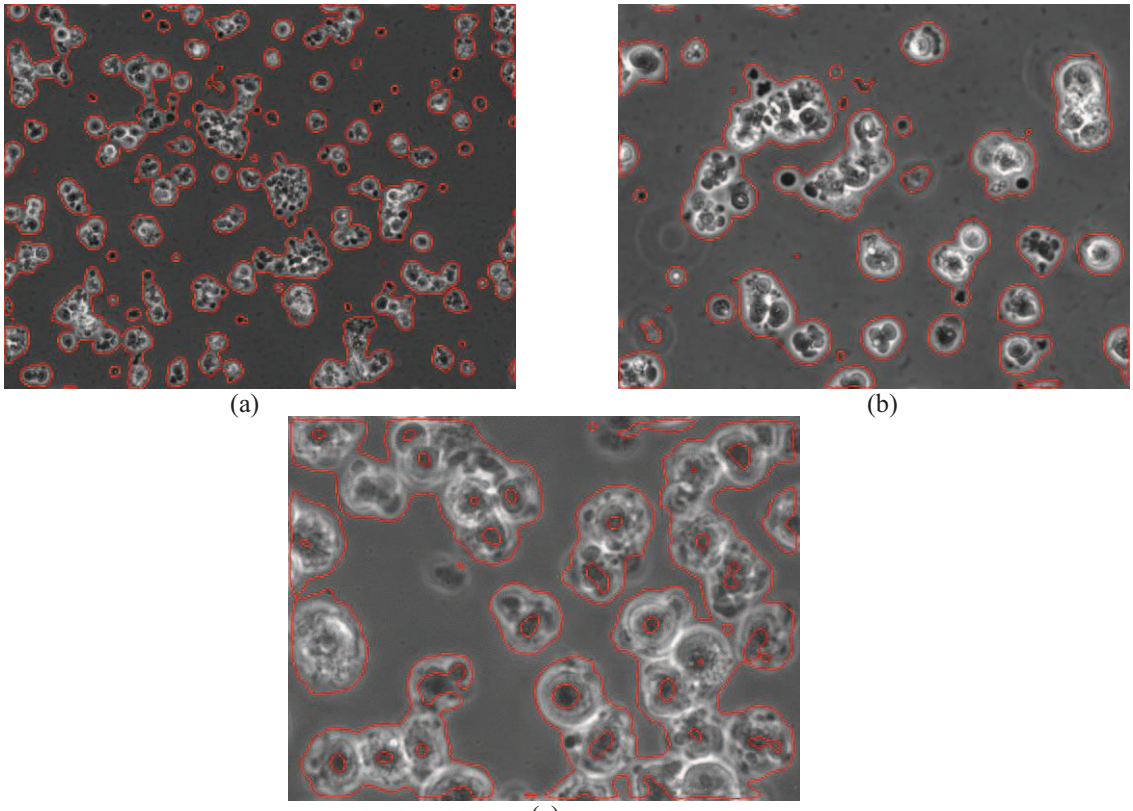
Figure 7: (a-c) K-means results for 10x, 20x and 40x.



(a) (b)

(c)

Figure 8: (a-c) Mixture of two Gaussians by EM, results for 10x, 20x and 40x.



(a) (b)

(c)

Figure 9: The results(a-c) of the proposed method for 10x, 20x and 40x respectively.

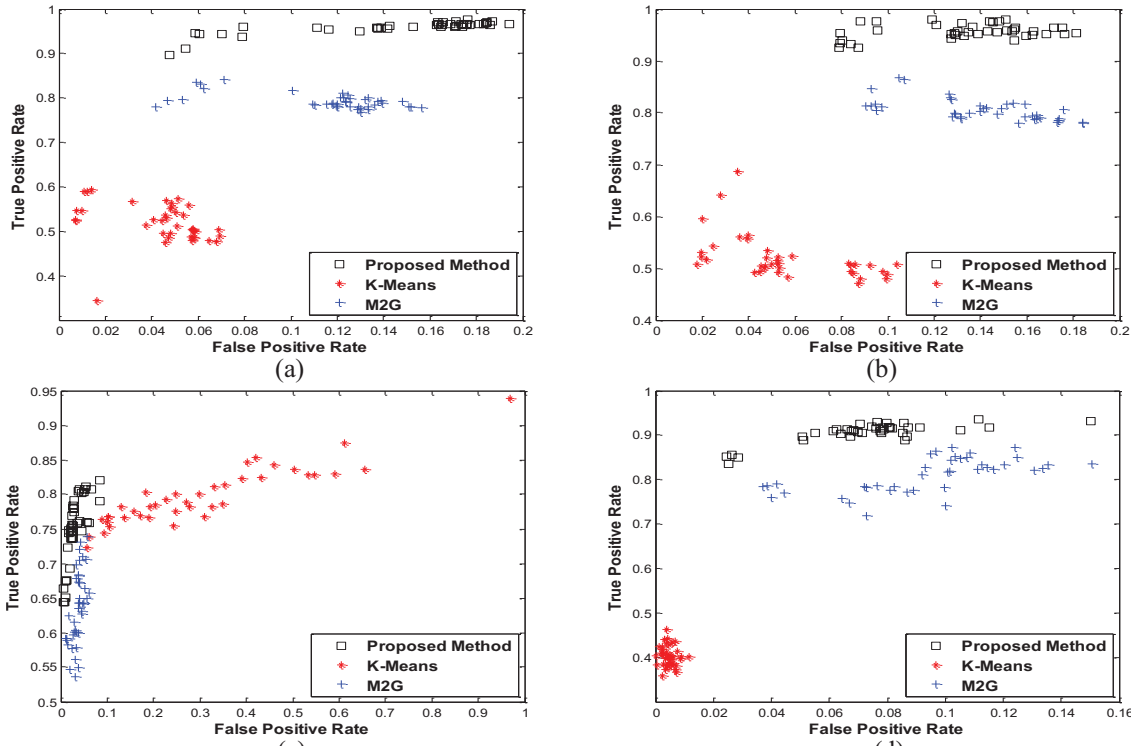


Figure 10: (a) The results of the video under 10x objective; (b) results of the video under 20x objective; (c) results of the noisy video under 40x objective; (d) results of the video under 40x objective after 5 x 5 median filter;

V. CONCLUSIONS

The proposed method incorporated the concept of spatial information and intensity distribution of the data for cell region detection. It uses the spatial information to improve the connectivity of local pixels to their corresponding data set. More importantly, it enables the fast convergence to the maximum absolute difference of foreground and background SNRs. The proposed method is able to split the image data into two Gaussian distributions; intensity distribution of the foreground and background data. Figure (10) shows that the proposed method achieves higher true positive rate. In case of noisy images, the pre-filtering of the image data can greatly improve the performance of the algorithm. In term of speed, the proposed method converges in less than 1.2 seconds while K-means and MGEM take about 3.61 and 25.3 seconds on a laptop with a Intel(R) Core™ 2 Duo CPU processor that run at 2.53GHz.

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