Expression of Antigens in Immunocytochemical Images Utilizing an Image Analysis Method

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Abstract— Immunocytochemistry is a common laboratory technique in which the quality of interaction between antibody antigen is evaluated on a cell surface. The amount of binding between antibodies and cancerous cells in Immunocytochemical images indicates how much the antibody is effective for treating the cancerous cell. In this paper, an automated system has been proposed for the first time, to measure such binding, utilizing well-known image processing methods. The experiments are simulated using the collected reference images in this research which can be used as a benchmark in the future researches. Proposing this method leads to an accurate and fast evaluation of the antigens’ expression along with more reduced human-error factors. (Abstract)

I. INTRODUCTION

Biomedical image processing has been turned into one of the most prominent theoretical and practical fields which is widely used for diagnosing and treating diseases. Breast cancer diagnosis and treatment are currently amongst the most vividly researched areas in biomedical image processing [1], [2], [3], [4] and [5]. The binding of antigens to antibodies are strongly specific like key and lock which make them useful for therapeutic. Immunocytochemistry is a common laboratory technique in which the quality of interaction between antibody-antigen is evaluated on the cell surface. These antibodies will be functional in diagnosis and therapy of cancerous cells. In the first step of production of antibodies, synthetic antigens are injected to mouse to trigger the natural immune system. After a couple of weeks, antibodies are extracted from the mouse’s blood. After extracting the antibodies and several other laboratorial processes, antibodies are added on fixed cancerous cells on slides, subsequently, an image is captured from cells by a fluorescent or light microscope for estimating the existence and amount of binding between the cells and antibodies which indicates how much the antibodies are effective for detecting and treating cancerous cells. Moreover, the numbers of binding sites leads to increase the affinity level of antibodies in a living organization [6], [7] and [8].

Up to now, no computer vision based approach has been proposed for analyzing Immunocytochemical images and this process is performed in manual manners which involves considerable human-error rate. In this paper, an automated approach is proposed for detecting a cell core along with calculating the amount of the antibody binding around the cell in Immunocytochemical images (refer to Figure. 1). Thus, the proposed method will have considerable importance in biomedical domain, regarding its collaboration in cancer treatment estimation by presenting flexible, fast and accurate expression of antigens, compared to manual process. Furthermore, the possibility of analyzing a wide range of antibodies for detecting the best antibodies will be provided in real-world applications.

In the proposed system, first of all, preprocessing methods are applied to Immunocytochemical still images for noise removing. Afterward classification and image enhancement methods in L*a*b color space are applied to Immunocytochemical images for separating the cell core from bindings to detect the cores as well as edge enhancements, respectively. Finally, the amount of binding between antibodies and cancerous cells are calculated utilizing some
index points in the cell circumference. The proposed method is evaluated utilizing 100 acquired Immunocytochemical images.

The rest of this paper is organized as follow: section II contains a description of principles and methods of the proposed system. Section III dedicates to the experimental results and their analysis. Section IV concludes the paper.

II. PROPOSED METHOD

The block diagram of the proposed system is shown in Figure. 2. In the first step of this system, dataset images are pre-processed for the noise removal. Subsequently, nearest neighbor classification method [9] is applied to the preprocessed images in order to separate cells’ core and bindings. In the image enhancement stage, the separated cells’ cores are enhanced in a different channel to be prepared for detection step. Finally, after detecting the cells, the amount of the binding is calculated. In order to evaluate the performance of the proposed method standard deviation metric is utilized and the error ratio is calculated. In the following parts of this section, the principles and methods used in the system are described.

A. Image Dataset

Up to now, there is no standard dataset containing Immunocytochemical images. In this research, we collected 100 Immunocytochemical RGB images for testing our methods. Three samples of these images are shown in Figure. 3. We collect variety types of these images to evaluate the method with high generalization. Since, the testing process has been manually performed in laboratory, the dataset images are evaluated by the experts and the percentage of the binding are calculated using average of the experts’ estimations as an evaluation metrics. Thus, results of the proposed methods will be compared with the experts’ subjective test. In addition, this dataset could be used as a benchmark in future researches in this domain, as well.

B. Preprocessing

The Immunocytochemical images which are captured by a microscope are considerably noisy. The noises are shown as random and banding noises which are reflected in a single pixel and whole image, respectively (Figure. 4-A). Random noises are reduced by smoothing spatial filters. It was observed in the course of experiment that uniform background has the capability of improving the accuracy. Therefore, for the sake of uniformity, morphological open operator is utilized to eliminate images’ background.

In addition, banding noises would be omitted as well. To be more specific, these noises are the effects of glass slides, and the lights reflected by a microscope during capturing process which increases a same level of illumination for the whole image. For removing this kind of noise, a statistical analysis has been done in noisy and noise-free images to calculate the mean and median of the red, green and blue channels. It could be seen that a noise-free Immunocytochemical image has the low mean in its red channel, regarding the lack of red color in these images (refer to Fig 1). In contrast, blue and green channels have the highest mean. The illumination which represents a binding noise is corresponded to the red channel. Thus, by subtracting the median of the red channel from all channels, the effects of noises are considerably reduced.

Figure. 4-B illustrates the image in Figure. 4-A which is preprocessed. As can be seen, the cells will be better detected and the images will be prepared for classification process.

C. Cell Core Localization

For locating the cells’ core and separating them from bindings, nearest neighbor classification method along with Euclidean distance metric is used in L*a*b* color image space, in which L, a and b indicate image contrast, red to green spectrum and blue to yellow spectrum, respectively [10]. An RGB Immunocytochemical image contains green and blue colors which sometimes they tend to phosphorus and violet colors, respectively. However, In L*a*b* color space, theses colors are highlighted in different channels. Thus, this is an appropriate color space on which the classification is performed. As can be seen in Figure. 5, nearest neighbor classification method separates the blue colors of cells’ cores and green colors of bindings in two different channels. To be illustrate, a sample of separated cells’ cores and bindings are shown are shown in Figure. 6-A and Figure. 6-B, respectively.
D. Image Enhancement in Frequency Domain

For enhancing the blue channel, including the cells, discrete Fourier transform is utilized. For this process, a high-pass filter with radius of 0.001 is used in frequency domain.

E. Cell and Edge Detection

In this research, a combination of Sobel and Log edge detection filters is used for detecting the cells. For this purpose, the results of Log filter applied to the core channel are used for calculating the threshold of the Sobel filter (Figure. 7-A). For better detection, Sobel filter is also used for improving the cells’ edge in 0 and 90 degree. According to this fact that the images are captured in different microscopes’ configuration, the size of the cells is different in various images. However, the size of the cells must be calculated for the next step. For master this problem, a pixel is selected inside the cell. Then, by moving toward neighbor pixels up to cells’ edge, the number of the pixel and subsequently the size of the cell are calculated. The size of the cells in Figure. 7-A, is calculated in Figure. 7-B.

F. Calculating Amount of the Connectivity

In order to calculate the amount of antibodies binding, two factors must be considered as follow: binding of the antibodies around the cell’s core and the diameter of the bound components. This process is performed in several steps.

Firstly, a number of index points in cells’ circumference is selected. To be more elaborate, the highest right point is selected in the cell’s area and other corresponding symmetric points are selected regarding the cell’s center. In Figure. 8, eight points are selected on the cell’s circumference. It is worth taking to consideration selecting more points leads to better calculation. The effects of selecting different number of points will be investigated in experimental results.

In the next step, a region is selected for each selected point. The size of this region is 0.125 of the cell’s area. The reason for this selection is that according to the experts’ opinion, this amount is adequate to be accepted as binding. In addition, due to this fact that different images are captured in different zooms, using a relative size leads to master the different zooms problem in this application. Then, a threshold is considered to accept the points as a bound component. To be more elaborate, the antibodies were separated in a different channel in the classification phase. Calculating the variance of the antibodies channel indicate that the amounts of this channel are not distributed around the mean. Thus, the threshold is calculated utilizing the first amount which is start of the fourth quarter of the channel’s amount in ascending order. The points which their values are more than the threshold are labeled as bound component. Afterward, number of the pixels in the bound components is divided to the number of pixels in the cells’ area in order to calculation the binding ratio.

Each image may involves several cells. Thus, the average of binding ratio for all cells in an image determines the amount of binding in the image. Figure. 9 shows results of applying proposed method to an image of the dataset.
III. EXPERIMENTAL RESULTS AND ANALYSIS

The proposed system is simulated using the dataset samples and the results of the proposed method are compared with the experts’ estimations. Error ratio and standard deviation metrics relate to the different number of the binding components are tabulated in Table 1. Error ratio metric indicates the average difference between the results of the proposed method and expert’s estimation and standard deviation shows variation of experimental results in comparison with the estimation of expert.

According to this fact that the antibodies which surround the cores affect the amount of binding, using more index points leads to less error ratio. The effect of utilizing different number of binding components is compared in Figure 10. It was observed in the course of experiments that utilizing eight points, which cover almost all of the binding around the cells’ circumference, can considerably reduce the error ratio.

Furthermore, the time consumed for the proposed method for each image is about 1 second, compared to the manual process in which each image averagely needs about 50 second to be evaluated. Above all, the significant amount of human-errors is prevented in all walks of this system.

It is worth taking to consideration the experts’ opinion is only an estimation (as the only valid metric in this domain) and marginal difference between the results of the proposed method and manual process does not show that the proposed method necessarily works better or worse, compared to manual process. Indeed, it shows that the proposed method performs correctly as much as manual process works. In addition to marginal error ratio, the slight amount of standard deviation reveals that the results are valid.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Number of connection components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Error Ratio</td>
<td>2</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>5.98</td>
</tr>
</tbody>
</table>

Figure 10. The effects of the number of selected index points on error ratio

IV. CONCLUSION

In this paper for the first time, an efficient and fast method was proposed for estimating the amount of antibodies binding using straightforward image processing approaches the outcome of which leads to positive effects on cancer treatment estimation. The proposed method was simulated using the collected Immunocytochemical images which could be used as a benchmark for future researches in this domain. The results of the proposed method reveal that there is no great deal of difference between utilizing the automated proposed method and manual process, where automated system works considerably quickly, and resistant in term of human error.

In the future works, we will survey the effects of the other classification together with feature extraction methods for improving the accuracy.

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REFERENCES


