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Image Enhancement Techniques for Automated Histopathological Analysis

Rucha Apte[1], Sanika Patange[2] , Ishan Joshi[3] , R. D. Komati[4] , Gurunath Kamble[5]
1 Department of Electronics and Telecommunication, MIT College of Engineering, Pune
2 Department of Electronics and Telecommunication, MIT College of Engineering, Pune
3 Department of Electronics and Telecommunication, MIT College of Engineering, Pune
4 Department of Electronics and Telecommunication, MIT College of Engineering, Pune
5 Optra Systems Private Limited, Pune

Abstract— Computer-assisted analysis of the tissue biopsy samples is used in order to reduce the time consumed by pathologists while determining the results manually. This has reduced the chances of inaccurate results due to human error thus improving accuracy achieved in detection and subsequent classification of a given sample. However, color consistency in light microscopy-based histology is an increasingly important problem with the advent of Gigapixel digital slide scanners and automatic image analysis. A variety of external problems such as weather, sunlight, dust, improper handling of the physical slides or noise introduced in the image can cause distortion of vital information. As a result, numerous image enhancement techniques have been developed to remove noise and enhance the images in order to recover the lost information. This paper provides a quantitative and qualitative analysis of a few image enhancement techniques applied on IHC stained TILs reinfused breast tissue images that use filtering and background correction using morphological structuring elements to rectify the distortion and provide enhanced images as an output which subsequently will give better results in detection and classification algorithms.

Index Terms— Histological Image Analysis, Color Image Enhancement, IHC Stained Images, Digital Pathology, Single Retinex, Rolling Ball Background Subtraction Method, Gaussian Method, Computational Biology, Computer-aided Diagnosis

1 Introduction

Serving as a gold standard of diagnosis for many diseases, histology is a critical and ubiquitous task in medical practice and research. Histopathology refers to the examination of invasive or less invasive biopsy sample by a pathologist under a microscope for locating, analyzing and classifying most of the diseases like cancer. [1] By automating routine histology analysis tasks, it is possible to reduce health care costs and improve diagnostic accuracy. Emergency situations, environmental noises, patients' special conditions in photography, lighting conditions and technical constraints of imaging devices are among the reasons why images may have low quality. [2-4] One challenge in automation is that histology slides vary in their stain intensity and color; we, therefore, seek a digital method to normalize the appearance of histology images. The use of digital pathology in medical image analysis has seen tremendous growth following the development of imaging technologies that can capture image data in-vivo as well as ex-vivo. Even though the field of radiology has adopted computer-aided image analysis in research as well as clinical settings, the use of similar techniques in histopathology is still in a nascent stage [5]. The pathologist examines the tissue structure, distribution of cells in tissue, regularities of cell shapes and determines benignly and malignancy in the image. The current gold standard in diagnosis involves labor-intensive tasks such as cell counting and quantification for disease diagnosis and characterization. This process can be subjective to human factors such as reader bias and fatigue. Therefore, a quantitative assessment of these images is very essential for objective diagnosis. Computer-based tools such as digital image analysis have the potential to help alleviate some of these problems while also give insights that may not be clear when viewing glass slides under an optical microscope. In this paper, we have used three methods; namely; gaussian method, single retinex method and the rolling ball method for performing image enhancement and background correction on immune-stained tumor infiltrating lymphocytes reinfused breast cancer tissue slides. The paper is organized to identify the accurate and robust method for performing color enhancement in IHC stained tissue images. In section 2, we have elaborated upon the related techniques carried to perform the image enhancement of the digitally acquired histology images. Section 3 of the paper gives an analysis of the techniques we have applied to IHC stained TILS reinfused breast tissue images. Section 4 of the paper gives a quantitative and qualitative analysis of the three methods used for image enhancement of tissue images. Finally, Section 5 concludes the paper and discusses the advantages of the proposed method and further lines of research. Supplementary materials provide an extensive additional evaluation.

2 Related Work

S. Waheed et al used a Gaussian filter set to the size of the cell nucleus to reduce the variations and perform smoothing operation. Even though the use of a Gaussian filter led to blur edges, it enhanced the segmentation results s compared to unfiltered counterparts. [6]

In order to improve the contrast and quality of medical images, Hamid Hassanpour et al proposed a method in which the disk-shaped mask along with Top-Hat transform was used as a structuring element that fitted the size of the original input image. In a multi-stage process, the size of this disk was increased at each step and the final enhanced image was used to determine the Contrast Improvement Ratio (CIR). This method showed a high potentiality to process a poor-quality medical image. [7]

Li X et al used an illuminant normalization module and a spectral normalization module to address the problem of color variation in histopathology images jointly caused by inconsistent biopsy staining and nonstandard imaging condition. This method produced stable and reliable color cues for stain normalization and insensitive to image content and achromatic colors. [8]

Calcification causes a major problem in X-Ray film for diagnosis of breast cancer. Hence, in order to overcome this drawback Zhang Q. et al proposed a medical image enhancement algorithm by adding details from the high-frequency subimages and decomposing the image especially with antisymmetric biorthogonal wavelet instead of some traditional wavelets. The method has many advantages in comparison with other methods, such as independent calcifications, low computational cost with impressive computing power and speed, clear edge contour and texture without agglomeration and big white chunks. [9]

In order to reduce the differences in tissue samples due to staining and scanning conditions, A. Can et al used color and illumination normalization. The illumination can be corrected either by using calibration targets or estimating the illumination pattern from a series of images by fitting polynomial surfaces. [10]

3 Proposed Work

3.1 Gaussian Method

The Gaussian method uses a Gaussian low pass filter that is applied to the separate RGB planes of the input color image. The result of applying a Gaussian filter is a blurred image, and on subtracting this blurred image we can perform background correction on the original input image. In order to reduce the rough margins caused due to variation in staining of the cellular features, images are smoothed with a Gaussian filter before segmentation is performed. Gaussian filter size is based on the size of the smallest feature of interest, the cell nucleus. The user retains the ability to adjust the size of the filter if necessary. Although the Gaussian filter tends to blur edges, an appropriately sized filter enhances segmentation results as compared to unfiltered counterparts, thereby validating the use of the filter. The background correction enhances the cells of the image, and this makes further processing such as segmentation and detection of cells easier. The Gaussian filter also reduces the difference in brightness between adjacent elements. It also can reduce blocking effects. However, we need to set the standard deviation of the filter to a custom value, which needs to be calculated. This can lead to suboptimal results if hit and trial method is used. [6]

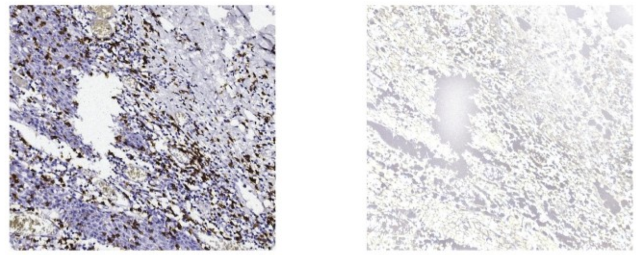


Fig. 1. Input Image and Gaussian Filter Output Image

3.2 Single Retinex Method

Edwin Land and McCann first proposed the retinex theory in 1964 [11]. Algorithms such as Multi-Scale Retinex (MSR) [12], Multi-Scale Retinex with modified color restoration (MSRCR) [13], Fast Multi-Scale Retinex (FMSR) [14] are based on Retinex Method. Retinex calculations aim to calculate the sensory response of lightness. A fundamental concept behind retinex computation of lightness at a given image pixel is the comparison of the pixel's value to that of other pixels. The main difference between the retinex algorithms is the way in which the other comparison pixels are chosen, including the order in which they are chosen [15]. In order to improve upon the dynamic range compression and preserve most of the details of the tissue cells, a single retinex method is used. In the single retinex method, the illumination is estimated by applying a Gaussian linear low pass filter to the separate RGB planes of the input color image. The output color image is obtained by subtracting the log signal of the estimated illumination, which is the 2D convolution of Gaussian surround function and original image for each of the RGB planes individually. The three planes are then recombined to get the enhanced image as the output. However, the single retinex method suffers from gray level violation problem and the images have a washed-out appearance.

Mathematically,

$$R(x1,x2) = \alpha(\log(I(x1,x2)) - \log(I(x1,x2) * F(x1,x2)) - \beta$$

Where,
R= Retinex Output
I=Input Image
F=Gaussian Filter
α= Gain Factor
β=Offset Factor

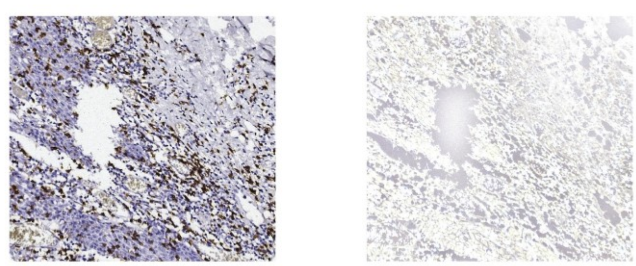


Fig. 2. Input Image and Single Retinex Output Image

3.3 Rolling Ball Background Subtraction Method

A local background value is determined for every pixel by averaging over a very large ball around the pixel. This value is thereafter subtracted from the original image, removing large spatial variations of the background intensities. The radius is set to the size of the largest object that is not part of the background. The rolling ball method uses a 2-Dimensional approach to manipulate the input image in a 3-Dimensional pattern. The working is similar to rolling a ball on a hand in a horizontal or vertical fashion. The rolling ball method is used for background subtraction and results in an enhancement in the cells for further processing. The rolling ball is implemented using the size of the cell in the slide as the radius for the ball and should be executed multiple times to achieve satisfactory results.

Mathematically,

Nx= -day/dr; Ny=dx/dr; Nz=0

where,

dx = displacement of input device in x-direction

by = displacement of the input device in the y-direction

dr = (dx2+dy2)1/2

We now introduce the single free parameter of the algorithm, the effective rolling ball radius R, which determines the sensitivity of the rotation angle to the displacement dr; for the size of our cells, the value of R is about 54.6.

The rotation angle is Θ, such that:

Cos Θ= R/(R2+dr2)1/2

Sin Θ= dr/(R2+dr2)1/2

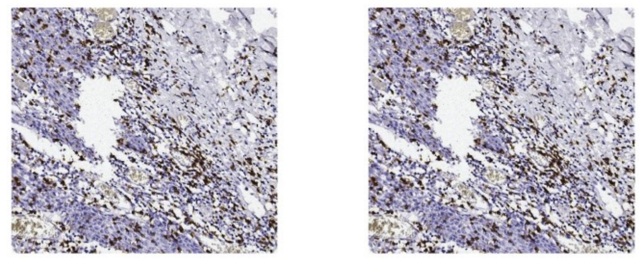
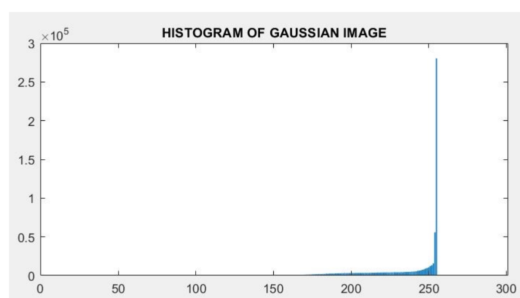
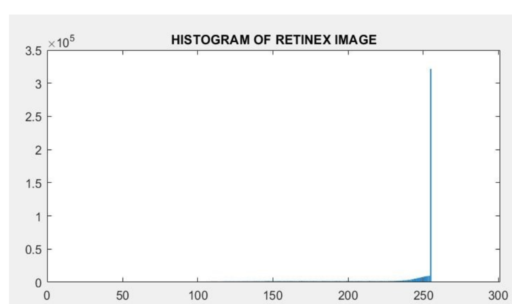


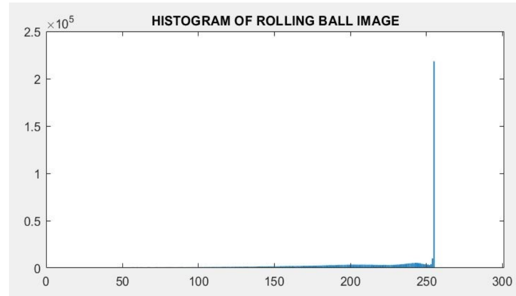
Fig. 3. Input Image and Rolling Ball Background Subtraction Output Image

4 Results and Analysis

The following table gives the PSNR values associated with the output images for each method as well as depicts a pictorial representation of the histogram for the output images.

TABLE 1
PSNR values and Histogram plots for output images

NO.	NAME OF THE METHOD	PSNR	HISTOGRAM
1	Gaussian Method	8.1147 dB	
2	Single Retinex Method	----	

3	Rolling Ball Background Subtraction Method	43.7457 dB	

5 Conclusion and Future Work

Thus, in this paper, we compare the results of using Gaussian filtering, Single Retinex and the rolling ball structural element on an image as image enhancement techniques. The filtering technique was shown to be the least effective, as it caused bleaching of the image. The Single Retinex method performs slightly better due to the theory of color constancy, but it still is not an optimal solution due to the color violation and rendition problems it poses in its output. The morphological structuring element or rolling ball method proves itself to be the most effective in terms of enhancement. The output of this method shows a much better qualitative as well as a quantitative result and does not have issues of bleaching or color violation as the other two methods do. A quantitative analysis of these methods based on their histograms and signal to noise ratios also shows the rolling ball method to be most effective. Image enhancement techniques provide a strong starting point to applications involved in the detection of certain important components of a biopsy sample or in the classification of a sample as testing positive or negative for the disease. They also help automate the process of counting or detecting certain features in samples which greatly reduces the human effort and chances of error that would otherwise be present in a manual process followed by pathologists.

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