Adaptive Color Illumination for Microscopes

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Abstract—Microscopes typically use a single color illumination source and the intensity of it can be varied depending on the application. They are used to observe various specimens which are usually stained to highlight the objects of interest. Automatic methods for identification of diseases from these specimens have been developed recently. The stains result in different colors and white illumination source may not offer the best contrast in all cases. Further, the staining process does not always result in the same color owing to the changes in the pH of the buffer used. This paper overcomes these problems by using a varying color illumination source for microscopes to offset the variations in the stain and ensures that the best contrast is achieved under all conditions. The results of segmentation of nucleus of White Blood Cells under varying color illuminations are shown as an example.

I. INTRODUCTION

Microscopic examination of specimens has been used for the diagnosis of several diseases. Microscopic observation of blood smears is considered the gold standard for malaria diagnosis [1]. Methods to automatically detect diseases from microscopic images taken from the specimens have been proposed recently [2], [3], [4], [5]. The performance of these schemes depends to a great extent on the contrast between the objects of interest and the background (or the rest of the objects). The specimens are usually stained to highlight the objects of interest. However, the staining process does not always result in the same color for the objects of interest and the background, owing to the variations in the pH of the buffer used (Fig. 1).

The performance of the automatic methods as well as that of manual observation can be improved if the variations induced due to staining are appropriately handled. It can be further improved if the contrast between the objects of interest and background is increased in addition to regular staining. There are several approaches to solve this problem. One solution would be to modify the acquired images to match the characteristics of a reference image. A two step procedure to perform color correction is presented in [6]. Optical lenses of microscopes generally pass more light in the center of Field of View being imaged and this is corrected using radiometric correction. The color properties are then corrected using gamma correction and color correction.

A color correction method for pathological slides prepared using inappropriate staining is described in [7]. The spectral transmittance obtained by a multispectral digital camera is used to estimate the amount of dye for each pixel. Then the amount of dye is adjusted based on the chemical kinetic equation by digital process so that the dye distribution is equivalent to that of the reference slide.

Several methods have been proposed to adjust the illumination of microscopes to suit the specimen being observed. Optical inverse tone-mapping is used to improve the visibility of specimens in [10]. The illumination is pre-modulated based on the local modulation properties of the specimen itself. The modulation of uniform white light by a specimen is estimated in real time, even though it is continuously but not uniformly illuminated. Microscopes usually have a photocell disposed in the optical path to detect the intensity of light being transmitted by an illuminator to a receptor plane. The light detected by the photocell is compared against a preset level of light in [11]. The intensity of illumination is adjusted automatically to a minimum level when the carrier is rotated and to a preselected level when an objective is in place for viewing.

Traditionally microscopes include a single color illumination source (typically white), the intensity of which can be adjusted to suit the specimen. Due to the recent advances in light-emitting diode (LED) technology, multicolor LEDs [12] have become affordable and are being regularly used for various applications. This paper proposes the use of a varying color illumination source for microscopes and the selection of optimal color for a given specimen using image processing methods.

Fig. 1. Malaria P. falciparum: Stages found in blood [8]

Fig. 2. Hue scale [9]
II. PROPOSED SCHEME

The color of illumination may have a significant effect on the contrast of the specimen. Hence we propose to change the color of illumination to suit the specimen under observation. The lamp of the microscope can be chosen appropriately if the application for which it is to be used is known a priori. However, a microscope is used to observe slides prepared from various tissues and changing the lamp before each observation may not be feasible. We propose the use of a Light Emitting Diode (LED) that can emit various colors to overcome this problem. We replace the regular lamp of the microscope [13] with an LED from Philips Color Kinetics [14]. However, it may be noted that an LED such as the one used here may not be the appropriate choice and that the intention here is to show that the proposed idea works. A camera [15] fitted to the c-mount of the microscope is used to record images.

Figure 3 shows the images captured under various color illuminations. As may be observed the contrast varied with the color of illumination. To show the effectiveness of the method, we demonstrate the segmentation of the nucleus of the White Blood Cells (WBC). Several schemes have been proposed for this purpose [16], [17]. It may be noted from Fig. 3 that the nucleus of WBC has a different color as compared to the rest of the WBC.

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Several schemes have been proposed to segment blood smear images. The green component of a peripheral smear image offers a good separation between the blood cells and the background and it also contains less noise. The histogram of the green channel is typically bimodal [18] and hence it is commonly used to segment blood cells [19]. We first segment the blood cells by thresholding the green component using an appropriate threshold [20]. The pixels belonging to each blood cell are given a unique label using blob coloring algorithm [21]. Then we convert those pixels belonging to the blood cells from RGB color space to HSV as it more intuitive for processing (Fig. 2) and process each blood cell independently. We divide the entire hue range of 360° into six segments where each segment has a width of 60° and is centered around a color type as given in Table I [22], [23]. Then we determine the dominant color type \( p \) by finding the number of pixels \( N_i \) that fall within each hue segment \( i, 1 \leq i \leq 6 \) and identifying the peak value \( N_p \) (i.e. \( p \) is associated with the mode of the hue distribution). The pixels not corresponding to the dominant color are identified as those belonging to the nucleus of WBC.

III. PERFORMANCE EVALUATION

The proposed scheme has been evaluated using images taken from several Leishman-stained blood smears. The input videos were in RGB color format and the dimension of each frame was 2048×1536 pixels. The results of segmentation under varying color of illumination are analyzed visually to determine the optimum color. The choice of color depends on the application and has to be chosen accordingly.

The results of the segmenting the nucleus of WBC in Fig. 3 (i.e., the subimage between coordinates (620, 260) and (850, 480)) are shown in Fig. 4. From these results it is clear that the segmentation of nucleus varied with the color of illumination and that it is best for the image in Fig. 3(a). The results for

<table>
<thead>
<tr>
<th>Hue Angle</th>
<th>Color Type</th>
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<tbody>
<tr>
<td>0°</td>
<td>red</td>
</tr>
<tr>
<td>60°</td>
<td>yellow</td>
</tr>
<tr>
<td>120°</td>
<td>green</td>
</tr>
<tr>
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<td>cyan</td>
</tr>
<tr>
<td>240°</td>
<td>blue</td>
</tr>
<tr>
<td>300°</td>
<td>magenta</td>
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Fig. 3. Frames of Sequence 1.
the images in Fig. 3(d), Fig. 3(f) and Fig. 3(g) are extremely poor because of the lower contrast between the nucleus and the remaining pixels of WBC. It is expected that the segmentation results obtained using other algorithms may also vary with the color of illumination. The results for Fig. 5 (subimage between coordinates (730, 1030) and (915, 1210)) are given in Fig. 6 and it can be seen that the best segmentation is obtained for Fig. 5(c). The images in Fig. 3 and Fig. 5 are taken from the same slide but the non-uniform staining that is inherent during slide preparation has resulted in a variation in the segmentation results.

IV. CONCLUSIONS

The single color source of illumination in microscopes, which is usually white, may not be suitable for all diagnosis applications as the color of the specimens varies depending on the preparation and the intended diagnosis application. We propose to vary the color of illumination depending on the specimen under observation to offset the variations in the staining so that the best contrast is obtained under all conditions. We achieve it by using an LED that can emit various colors. An application of the method to segmentation of nucleus of WBC is shown and the results show that the best contrast is obtained when the optimal color of illumination is used.

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REFERENCES


Fig. 5. Frames of Sequence 2.

Fig. 6. Segmentation Results for Frames of Sequence 2 (Fig. 5).