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Review

Digital image analysis for automatic enumeration of malaria parasites using morphological operations

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ABSTRACT

Every year, malaria kills between 660,000 and 1.2 million people, many of whom are children in Africa. The World Health Organization (WHO) encourages the development of rapid and economical diagnostic tests that allow for the identification of proper treatment methods. In this paper a novel method to automatically enumerate malaria parasites is proposed and evaluated, using a database consisting of 475 images with varying densities of malaria parasites. This method will analyze data by utilizing standard operations of image processing such as histogram equalization, thresholding, morphological operations and connected components analysis for parasite density estimation. The application of the proposed method yields an average accuracy rate of 96.46% with a low processing time of two seconds per image on a custom computing platform.

1. Introduction

Malaria has infected humans for over 50,000 years (Day, 2010). Epidemiological data show that somewhere in the world a child dies every 30 s because of this disease. This suggests that both vaccine development and the proper administration of available drugs are absolutely essential to treat malaria patients (O'Meara, Hall, & McKenzie, 2007a). A wrong diagnosis of this disease may have adverse clinical and therapeutic implications for patients and for endpoints of clinical trials of anti-malarial vaccines or drugs. Nowadays, there are a lot of different techniques for malaria diagnosis available in the market (Bhandari, Raghuveer, Rajeev, & Bandhari, 2008; Ngasala et al., 2008; Tangpukdee, Duangdee, Wilairatana, & Krudsood, 2009), but conventional microscopic examination remains the gold standard (Frean, 2010; Warhurst & Williams, 1996; Wongsrichanalai, Barcus, Muth, Sutamihardja, & Wernshorfer, 2013). Thus, the most important component of laboratory diagnosis is the quantification of parasite density. Accurate and replicable parasite counts are difficult to achieve because of inherent technical limitations and human inconsistency (Mitiku, Mengistu, & Gelaw, 2003). Digital image analysis provides an opportunity to improve the performance of parasite density quantification, decreasing the time required to count the parasites and avoiding bias from human error.

A technique was proposed (Díaz, González, & Romero, 2009) for quantifying erythrocytes in stained thin blood films. The image was corrected from luminance differences and the normalized RGB color space was used for classifying pixels as erythrocyte or background followed by an Inclusion-Tree representation that structures the pixel information into objects. Finally, a two step classification process identifies infected erythrocytes, using a trained bank of classifiers. The main drawback of this approach is that it needs user intervention, which results in time consuming and large inter and intra-observer variabilities (Alexander, Schellenberg, Ngsala, Petzold, & Drakeley, 2010).

Automatic parasite detection has been addressed in Abdulnasir, Mashor, and Mohamed (2013), Das, Ghosh, Pal, Maiti, and Chakraborty (2012), Frean (2009), Moon et al. (2013) and Tek, Dempster, and Kale (2006, 2010). In Makkapati and Rao (2009), a scheme based on HSV color space is presented. This method was focused on detecting dominant hue range and calculating optimal saturation thresholds. The dominant color in this kind of images is representative of the background, so dividing the hue range of 360° into six 60° segments enables to find the number of pixels that fall within each hue segment and thus, the dominant range of color. Optimal saturation thresholds were identified by using the method proposed in Otsu (1979). This automatic thresholding method is

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widely used in classic image segmentation applications (Guo, Wang, & Xia, 2014; Vala & Bashi, 2013; Yang, Shen, Long, & Chen, 2012) and is based on selecting the discriminant criterion, so as in order to maximize the separability of the resulting classes in gray levels. This technique was found to give an optimal threshold for bimodal distributions but did not work well for unimodal distributions. Precisely, the blood smear images present unimodal distributions due to the fact that most of the pixels belong to the background, and only a little bit of them are pixels from parasites, resulting in a histogram with only a big and wide peak.

In further studies, granulometric estimation and morphological techniques were employed with promising outcomes (Khatri, Ratnaparkhe, Agrawal, & Bhalchandra, 2013). A method (di Ruberto, Dempster, Khan, & Jarra, 2001) introduced a morphological approach to cell segmentation, which was more accurate than the classical watershed-based algorithm (Bieniek & Moga, 2000). A hemispherical disk-shaped structuring element was used to enhance the roundness and the compactness of the red cells, while a disk-shaped flat structuring element was used to separate overlapping cells. Regarding the classification step, two different methods were used, one based on morphological operator (Díaz-Huerta, Felipe-Riveron, & Zetina, 2014; González & Woods, 2002) and another one based on color histogram similarity (Colombo, Rizzi, & Genovesi, 1997; del Bimbo, Mugnaini, Pala, & Turco, 1998). Despite the brilliant solutions presented by these approaches, these kind of techniques were very sensitive to the image quality. Besides, the fact that it is necessary to analyze both the hue and the saturation images makes the process slow down, although morphological operations will be used by our approach in a different way.

In this study an image processing technique, which is not used on conventional algorithms, is proposed, focusing on a low time consumption and obtaining good results independently of the variability of the images. Fig. 1 shows a block diagram of the proposed algorithm for enumerating parasites. Firstly, the acquired image (a gray-scale image, not an RGB or HSV) is preprocessed by means of a low pass filtering that removes noise, and an adaptive histogram equalization that corrects the contrast of the images to improve its quality. Once this is achieved, the adequate threshold to binarize the image is estimated. In our method, an adaptive thresholding based scheme allows an effective classification of pixels. This means that the election of whether a pixel belongs to the background or to the signal (parasites and white blood cells) is only established by the pixels around it, that is its neighborhood. Then, morphological methods (regional minimum and closing operation) use granulometries to evaluate the area of the connected components, labeling the components belonging to parasites and counting the number of them.

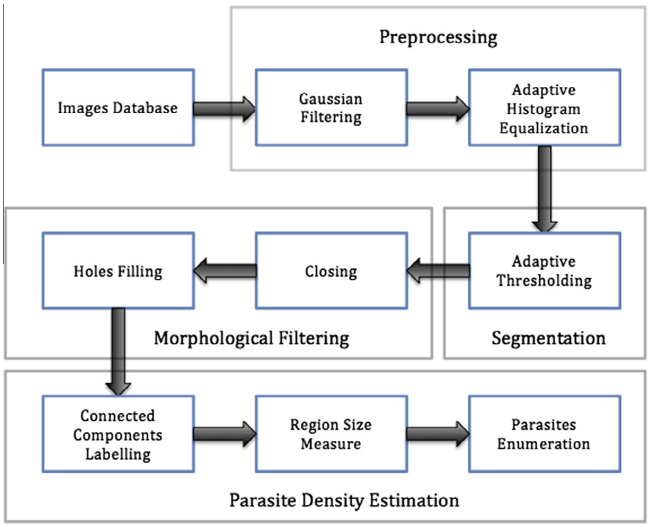

Fig. 1. Image processing steps.

The organization of the rest of the paper is as follows. Details or our method based on adaptive processing and morphological operations are mentioned below. The method consist of four stages. In Section 2.1 the preprocessing step is explained. This consists of a Gaussian low-pass filter to reduce the noise of the image and an adaptive histogram equalization with the objective of achieving an image with more contrast. Then, in Section 2.2 we focus on an adaptive thresholding process that allows an automatic binarization of an image dividing the entire image into small subimages and applying different equalizations to each one. Section 2.3 explains different operations based on the so-called mathematical morphology. These operations are capable of filling the holes in parasites created in previous steps and eliminating all the elements in the image that are smaller than the structural element. In Section 2.4, a measurement of the properties of the regions are made, resulting in labeling and subsequent classification (parasites or white blood cell) as based on this measure. The experimental results are provided in Section 3, and a discussion of research contributions and practical advantages in addition to the conclusions are available in Section 4.

2. Image processing

2.1. Preprocessing

The aim of the preprocessing stage is to obtain images with more contrast than the original image. This process consists of two operations to increase the contrast of the raw acquired images: image filtering to mitigate noise and image enhancement, in the form of histogram equalization. In this paper, we are interested in filtering operations that are performed directly on the pixels of an image. That is why the term *spatial filtering* is used to differentiate this type of process from the more traditional frequency domain filtering. Generally, the linear spatial filtering of an image f whose size is $M \times N$, using a $m \times n$ mask, is given by the following function:

$$g(x,y) = \sum_{s=-a}^{a}\sum_{t=-b}^{b} w(s,t) f(x+s, y+t) \tag{1}$$

where ω's are mask coefficients. To generate a completely filtered image, this equation must be applied to $x = 0, 1, \ldots M-1$ and $y = 0, 1, \ldots, N-1$, where $a = (m-1)/2$ and $b = (n-1)/2$.

In this stage, a Gaussian low-pass filter is applied to the input image to get clear signal regions and suppress the influence of noise. Fig. 2 shows the result of convolving a Gaussian mask with the original image. After Gaussian filter, the image is brighter than the original one, thus the intensities of the pixels are higher than they were before. This means that the process of choosing a threshold

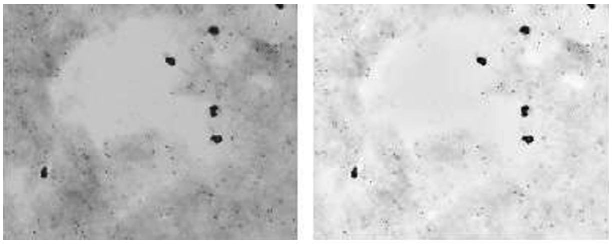

Fig. 2. Left side: original image. Right side: image after Gaussian Filtering.