Micron 65 (2014) 20–33

Contents lists available at ScienceDirect

Micron

journal homepage: www.elsevier.com/locate/micron

Review

Automated microscopic image analysis for leukocytes identification: A survey

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ARTICLE INFO

Article history:
Received 7 January 2013
Received in revised form 30 January 2014
Accepted 1 April 2014
Available online 12 April 2014

Keywords:
Image preprocessing
Leukocytes segmentation
Feature extraction
Feature selection
Classification

ABSTRACT

Automatic quantification and classification of leukocytes in microscopic images are of paramount importance in the perspective of disease identification, its progress and drugs development. Extracting numerical values of leukocytes from microscopic images of blood or tissue sections represents a tricky challenge. Research efforts in quantification of these cells include normalization of images, segmentation of its nuclei and cytoplasm followed by their classification. However, there are several related problems *viz.*, coarse background, overlapped nuclei, conversion of 3-D nuclei into 2-D nuclei etc. In this review, we have categorized, evaluated, and discussed recently developed methods for leukocyte identification. After reviewing these methods and finding their constraints, a future research perspective has been presented. Further, the challenges faced by the pathologists with respect to these problems are also discussed.

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1. Introduction

Image analysis and pattern recognition methods have been used extensively in the field of pathological analysis to assist pathologists in studying different patterns/ cells in the microscopic images. There are different types of cells available in the diagnostic smear (blood smear, impression smear, etc.) or tissue sections. These include red blood cells (RBC), white blood cells (WBC), platelets, tissue specific or transmigrated cells, and combination of these cells. Out of these cells, white blood cells, also known as leukocytes or inflammatory cells, are the cells of the immune system which defend the body against infectious disease and foreign materials (Kumar et al., 2010). Quantifying leukocytes as a part of defining their role in disease process (Lomash et al., 2013; Agrawal et al.,

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http://dx.doi.org/10.1016/j.micron.2014.04.001
0968-4328/

2012; Gupta et al., 2010) or during screening of anti-inflammatory drugs (Lomash et al., 2011) is of immense importance. Moreover, the classification of cells is essential to know the type and duration of inflammation process which can also be helpful in clinical therapeutic aspects. Therefore, a review of recently available image analysis methods for leukocytes quantification in digitized images is being presented here for diagnostic and research purpose.

During inflammation, leukocytes are migrated into tissue sections from blood vessels to wall off the injurious agents and start the healing process (Lomash and Pant, 2014). Leukocytes are divided into two main categories as per the structure of nuclei: granular (polymorphonuclear cells) and non-granular (mononuclear cells). Granulocytes have granules in their cytoplasm and they are of three types: neutrophils, basophils, and eosinophils while lymphocytes and monocytes cells are the types of non-granular cells which have single nucleus. Leukocytes have no color, but they can have color when stained with chemicals to make them visible under the microscope (Kumar et al., 2010).

Despite the increasing sophistication of modern diagnostic tools, pathologic anatomy is still the principal means by which most diagnosis proceeds (Jones et al., 1997). Recently, some work has been presented for manual quantification of leukocytes on tissue sections either for explaining leukocytes' role in disease process (Lomash et al., 2013; Agrawal et al., 2012; Gupta et al., 2010) or for screening anti-inflammatory drugs (Lomash et al., 2011). However, manual counting of leukocytes have some major problems like, it is a time consuming process, requires a dedicated trained pathologist, the individual microscopic observations are biased in nature due to presence of wide natural biological variability across tissue sections, and pathologists generate qualitative not quantitative assessment of disease (Gurcan et al., 2009).

These problems may be reduced by the automated quantification of leukocytes. A validated automatic counting and classification system for the leukocytes can provide the quantifiable data more accurately and quickly as compared to manual analysis. Recent advances in image analysis and pattern recognition open up the possibilities of automatic detection and classification of leukocytes. Automatic leukocytes detection could bring the efficacy in analysis report in terms of time and accuracy which will be unbiased from human expertise. Such a system could also make classification of different types of cells as a powerful tool for researchers in life science and medicine. Number of methods have been developed for identification of leukocytes in past two decades. Some review papers focusing on different aspects of blood smear and tissue section images are published in recent past (Gurcan et al., 2009; Demir and Yener, 2005; Ong et al., 1996; Buttarello and Plebani, 2008), but the review literature on the state-of-the-art techniques for leukocytes identification is meagre. Therefore, this paper reviews various leukocytes detection methods along with their constraints. A future research perspective for leukocytes identification has also been presented.

Rest of the paper is organized as follows: Section 2 describes the techniques presented in the literature for leukocytes segmentation and classification. Possible directions for future research are discussed in Section 3. Section 4 concludes the paper.

2. Literature review

The role of computer-based image analysis systems in the field of quantitative histopathology and cytology are reported as early as 1960 (Ong et al., 1996). Most of the early work concentrated on blood smears (Prewitt and Mendelsohn, 1966), chromosomes (Castleman et al., 1976) and cervical smears (Diacumakos et al., 1962). Unlike analysis of cytological images, tissue images pose more complexity due to wide variety of overlapping cells and coarse background (Gurcan et al., 2009; Ong et al., 1996). The process of automatic classification of leukocytes can be divided into four steps (Gurcan et al., 2009): (i) image preprocessing, (ii) leukocytes segmentation, (iii) feature extraction, and (iv) leukocytes classification. These processes are discussed individually in the following sections.

2.1. Image preprocessing

To highlight the colorless leukocytes available either in tissue section or blood smear, special type of dyes/chemicals are used and this process is known as staining. Different types of methods used for staining are: Wright's stain (Pan et al., 2012), Pappenhein stain (Wermser et al., 1984), Maygrinwald giemsa (Hamghalam et al., 2009), Leishman (Ghosh et al., 2010), hematoxylin-and-eosin (H&E) (Kuse et al., 2010), immunohistochemically (IHC) stain (Kraan et al., 2000), immunofluroscence (IF) stain (Nattkemper et al., 2001), etc. Photomicrographs of tissue section or blood smear may have variations in their color intensity due to concentration of staining, aging of the staining solution/stained slide, etc. The images of blood smear or tissue sections for clinical and preclinical analysis are widely acquired through bright-field microscopy. The quality of images are also affected by the use of various types of illuminators such as LED, HBO, and XBO (Bradbury and Bracegirdle, 1998; Pluta, 1989, 1988; Gretz and Duling, 1995; Hammersen and Duling, 1980). Images are further affected by the exposure time and types of camera lenses such as 0.7 NA air lens, plan apochromatic lens, and lenses with chromatic aberrations (Arce et al., 2013; Light Microscopy, 2013). However, to highlight the features not visible under white light, colored or polarized filters are used on the light source (Bradbury and Bracegirdle, 1998; Pluta, 1989) but color and illumination variations are still observed and require image preprocessing before segmentation. An example of such variations of different organs and blood smear images is shown in Fig. 1. All the representative images are stained using hematoxylin-and-eosin (H&E) staining. The images are taken from Histopathology Section of Defence Research & Development Establishment, Gwalior, India. All the photomicrographs were acquired using a DC500 camera (Leica, Wetzlar, Germany) attached to DMLB microscope (Leica) at 40× magnification.

The color and illumination variations can reduce the efficiency of manual or automated identification system which may lead to biased analysis. Therefore, these images must be normalized to minimize the variations. This facilitates the better segmentation and consequently improving the classification accuracy (Gurcan et al., 2009; Ong et al., 1996). A number of leukocytes classification methods are proposed on gray scale images which remove the need of color normalization but lead to the loss of color information of the cells. Further, little work has been done for color normalization in the field of microscopic images for H&E stained images (Macenko et al., 2009; Magee et al., 2009; Niethammer et al., 2010). Different color normalization methods used for H&E stained microscopic images are illustrated in Table 1.

In general, the color normalization methods are based on either color transfer methods or deconvolution-based methods. Color transfer is one of the prime methods, used in recent years for changing the color appearance of an image (Reinhard et al., 2001; Abadpour and Kasaei, 2004; Tai et al., 2005). The concept of color transfer is introduced by Reinhard et al. (2001) by incorporating the color of reference image into source image. Liu et al. (2012) corrected the color variation within the images, stained with H&E staining, using the color transfer method proposed by Reinhard et al. (2001). The color transfer method of Reinhard et al. (2001) is based on matching of mean and standard deviations of each color channel of source and target images. As all the three channels R, G, and B of RGB color space are correlated with each other,