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Leukocyte segmentation in tissue images using differential evolution algorithm

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ABSTRACT

An automatic segmentation of leukocytes can assist pharmaceutical companies to take decisions in the discovery of drugs and encourages for development of automated leukocyte recognition system. Segmentation of leukocytes in tissue images is a complex process due to the presence of various noise effects, large variability in the images and shape of the nuclei. Surprisingly, rare efforts have been made to automate the segmentation of leukocytes in various disease models on hematoxylin and eosin (H&E) stained tissue images. The present work proposes a novel strategy based on differential evolution (DE) algorithm to segment the leukocytes from the images of mice skin sections stained with H&E staining and acquired at 40 × magnification. The proposed strategy is a first inline report used in such type of image database. Further, the proposed strategy is compared with well-known segmentation algorithms. The results show that the proposed strategy outperforms the traditional image segmentation techniques.

1. Introduction

Inflammation is a complex protective reaction where injurious agents are either destroyed, diluted or walled-off [1]. Inflammation nearly occurs in every known disease at some time in their course. A controlled inflammatory reaction is a part of protective mechanism; however, in many instances identification and removal of inflammation are not achieved. This reaction is very deleterious. A number of anti-inflammatory drugs are available [2] but due to their reported harmful side effects in recent posts, some of them are banned in market for further use (e.g. Rofecoxib). Thus still there is a challenge for scientists to discover anti-inflammatory drugs with minimal side effects.

Inflammation has two phases: exudative (acute inflammation phase) and cellular (sub-acute or chronic phase) [1]. A variety of instruments are available to aid automation of acute inflammatory reactions for reducing human subjectivity and workload. However, rare work have been done in the quantification of inflammatory cells in the tissue images due to their wide natural biological variability. Recently, we have noticed articles on chicken skin model [2], mice skin model [3], air pouch model [4], etc. where inflammatory cells (leukocytes) were quantified manually. Manual counting of leukocytes is a time consuming process and requires a trained dedicated pathologist. The number of variables such as chances of biasness and variations in staining characteristics pose challenges for the research scientists in counting leukocytes manually [5,6]. Therefore, to reduce these problems, there has been a growing interest in developing tools for quantification of leukocytes using image processing techniques. Image processing techniques are widely used in the field of medical imaging, computer vision, security, etc. [7–9]. Therefore, it is interesting to use the image processing techniques for quantifying the leukocytes. Some useful algorithms have already been developed for separation of leukocytes in blood smears [10,11] but rare efforts have been made for tissue section images [6] due to their complex structural morphology.

Leukocytes consist of nucleus (bluish color when stained with hematoxylin and eosin (H&E) staining) within cytoplasm (pinkish color). Leukocyte can be identified with the structural and textual information of its nucleus and cytoplasm. Therefore, nuclei and cytoplasm segmentation have vital importance in leukocytes identification system. Automation of leukocytes segmentation faces a set of challenges such as shape and size variability and presence of different artifacts [5,6], etc. Further, the tissue images stained with H&E staining make the segmentation process more complex. Therefore, considering the above limitations, a novel leukocytes segmentation strategy for images of inflamed mice skin section stained with H&E staining and acquired at 40 × magnification using differential evolution (DE) algorithm is proposed.

DE [12] is an evolutionary algorithm (EA) and has a vital difference from other evolutionary techniques like genetic

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algorithms (GA) [13]. DE has a prime ability to explore the large search spaces efficiently. Two major advantages of DE are that it has a faster convergence rate as compare to other evolutionary algorithms and requires to adjust a few parameters due to which it can be implemented easily [14]. Recently, Das and Suganthan [15] presented state-of-the-art of DE and its major variants along with its applicability to a larger set of applications. During the last decade, the different variants of DE includes JADE [16], SaDE [17], DEGL [18], jDE [19], etc. Recently, Islam et al. [20] proposed MDE_*p*BX and observed that by applying proposed modification, the ability of DE can be improved for finding a solution in a given search space. Furthermore, the solution search capability of advance DE variants, like jDE and JADE, can also be enhanced by incorporating their proposed mutation, crossover, and parameter adaptation schemes.

Vesterstrom and Thomsen [21] observed that DE outperforms particle swarm optimization (PSO), and EAs regarding their general applicability as numerical optimization techniques using 34 widely used benchmark problems. Price et al. [12,14] compared DE with simulated annealing, variants of GA, and other evolutionary programming and observed that DE is more accurate and efficient. Hammouche et al. [22] presented a comparison among six meta-heuristic techniques namely GA, PSO, DE, ant colony optimization (ACO), simulated annealing (SA), and tabu search to solve the multilevel threshold problem of image processing and found that DE provides the most efficient quality solutions. Osuna-Enciso et al. [23] presented an empirical comparison of three state-of-the-art-nature inspired algorithms, PSO, artificial bee colony (ABC), and DE to perform image threshold by a mixture of Gaussian functions. They used statistical analysis tools to compare the results and concluded that DE not only shows a superior performance in minimizing the Hellinger distance between the original and the candidate histogram but also performs minimization in less number of evaluations for the mentioned distance cost function. Iwan et al. [24] presented a comparative study for min–max constrained optimization using PSO and DE using some selected standard benchmark functions and observed that DE outperforms PSO in terms of repeatability and quality of obtained solutions. Das et al. [25] showed the superiority of DE by extensively comparing the automatic clustering DE (ACDE) with two other state-of-the-art automatic clustering techniques based on GA and PSO. Further, DE has been successfully applied to a diverse set of optimization problems [26–32].

Therefore, this paper presents a novel leukocytes segmentation method based on differential evolution (DE) which extracts out the leukocytes from the images of inflamed mice skin section stained with H&E staining and acquired at 40 $\times$ magnification. The results are compared with well-known segmentation algorithms used by different researchers for leukocyte segmentation namely *K*-means [33], expectation–maximization (EM) algorithm [34], Fuzzy *c*-means [35] and Otsu's threshold [36].

Rest of the paper is organized as follows. Section 2 describes the problem formulations and its description. The differential evolution algorithm (DE) is explained in Section 3. The proposed segmentation strategy is described in Section 4. In Section 5, experiments are performed to compare the performances of the proposed strategy with traditional well know strategies. Finally, in Section 6, paper is concluded along with discussion.

2. Problem description

The image segmentation is a process of partitioning an image into multiple regions based on selected image properties like color, area, shape, etc. In this paper, we are dealing with the problem of automated segmentation of leukocytes in mouse skin section images stained with H&E staining. Segmentation of leukocytes in tissue images is a complex process due to the presence of various staining, illumination, and structural variability along with different artifacts/noise. Therefore, to segment these complex images, the use of multilevel image segmentation is appropriate as compare to single level. In this paper, a multilevel image segmentation technique has been introduced based on differential evolution (DE) algorithm [12].

DE [12,37] has successfully been applied to the applications of image segmentation [38] and pattern recognition [39]. This paper describes the use of DE algorithm for segmenting the leukocytes from the images of inflamed mice skin section stained with H&E staining. Since the preparation of a glass slide of tissue section undergoes various processing phases like sectioning, staining, etc., some artifacts/noise may be introduced in the slide that need to be differentiated from the leukocytes. These artifacts/noise are also visible in the microscope and have almost similar intensity as that of leukocytes. These unwanted objects could only be distinguished from the leukocytes on the basis of nucleus and cytoplasm features not by their intensity. Therefore, in the proposed mechanism, the leukocytes segmentation is done in two phases. In the first phase, objects of interest are segmented from photomicrographs of inflamed mice skin using pixel intensity information. These set of objects may also contain artifacts which are minimized in the subsequent phase. In the second phase, segmentation is based on the feature set (area of cell, eccentricity of cell, ratio of cytoplasm area to nucleus area, solidity of nucleus) of the extracted objects. The algorithm made no priori assumptions about the size, shape, texture or spatial distribution of the leukocytes.

The problem of leukocytes segmentation can be treated as an optimization problem defined by an appropriate objective function. The objective functions designed to solve this type of complex problems are generally complex, multimodal, and combinatorial and cannot be bounded by mathematical forms. Therefore, the application of several traditional strategies becomes limited. Meta-heuristic techniques are not bounded by these restrictions and hence can be a better choice to solve these types of problems. The formation of the objective functions to deal with both the segmentation phases for the proposed strategy are described in following sections.

2.1. Intensity based segmentation

The mathematical formulation of the intensity based segmentation technique is inspired by the strategy presented in [40]. Let a given RGB image, having N number of pixels, consists of L intensity levels ($\{0,1,2,\ldots,L-1\}$) for each RGB plane and n_i represents the number of pixels of intensity level i. Then, the probability of an occurrence of a pixel of intensity level i in the image, also known as probability distribution (p_i), is given as

$$p_i = \frac{n_i}{N}, \quad 0 \le i \le L-1. \tag{1}$$

The mean intensity of each RGB plane of the image is determined by the following equation:

$$\mu = \sum_{i=1}^{L} i p_i. \tag{2}$$

To classify the pixels of the image into n classes $\{D_1,D_2,\ldots,D_n\}$, $n-1$ thresholds $(t_1,t_2,\ldots,t_{n-1})$ are to be generated as expressed in