

# Blood Cell Segmentation: A Review

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**Abstract - The analysis of blood cells in microscope images can provide useful information concerning the health of patients; however, manual classification of blood cells is time-consuming and susceptible to error due to the different morphological features of the cells. Therefore, a fast and automated method for identifying the different blood cells is required. In this paper, we propose different methods to segment Red Blood Cell (RBC), White Blood Cell (WBC) and Platelets. Image segmentation is the most important step and a key technology in image processing which directly affect the next processing. In human blood cell segmentation cases, many methods were applied for obtaining better results. In this paper, we review some of the general segmentation methods that have found application in classification in biomedical-image processing especially in blood cell image processing. Basically, segmentation of the image divides the whole image into some unique disjoint regions. The fact that the segmented image should retain maximum useful information and discard unwanted information makes the whole process critical.**

**Keywords- Image segmentation, Image processing, Blood segmentation, Blood Cells and White Blood Cell.**

## I. INTRODUCTION

The use of image processing helps to improve the image quality and analysis approach from different application. It improves the effectiveness of the analysis in term of accuracy, time consuming and so on [1]. Human blood is composed mainly of three main cell types, White Blood Cells (WBCs), Red Blood Cells (RBCs) and platelets.

The counting of these blood cells is known as a complete blood count and provides information such as the lack or over abundance of certain cells which could indicate certain diseases such as leukemia or anemia. WBCs in particular can help to determine the state of health of a person as well as some diseases they may have. The reason for this is that WBCs are produced as a reaction to illness. Overproduction or underproduction can also indicate certain diseases including infections, allergies, blood related conditions and the body's

response to treatment. The number and shape of the RBCs can also indicate certain medical problems. Since the RBC carries oxygen from the lungs and carbon dioxide to the lungs, the RBC count is a great indicator to determine the body's oxygen level [2].

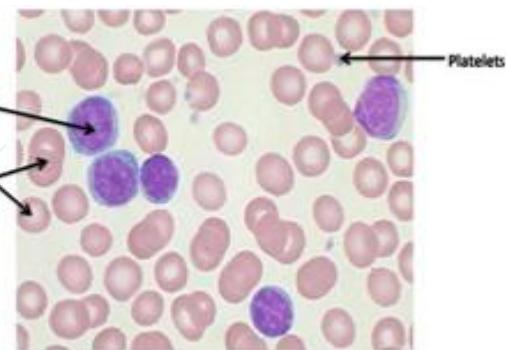


Figure 1 An image of blood smears showing red blood cells, white blood cells and platelets

Cell segmentation is a challenging problem due to both the complex nature of the cells and the uncertainty present in video microscopy. Manual methods for this purpose are onerous, imprecise and highly subjective, thus requiring automated methods that perform this task in an objective and efficient way. One of the most important part in image processing is segmentation. Recent studies have suggested different method for segmentation and identification of blood cell.

## II. SEGMENTATION TECHNIQUES

Segmentation is one of the major problems in image analysis and considerable research has been performed in trying to solve this problem including methods of segmenting various types of cells or biological images. In computer vision, segmentation refers to the process of partitioning a digital image into multiple regions. The goal of segmentation is to simplify and/or to change the representation of an image into something that is more meaningful and easier to analyze [3]. Some of the practical application of image segmentation are used in medical imaging to study of anatomical structure,

diagnosis, treatment planning or locate tumors and other pathologies [4]. Other than that, this application is also used in face recognition, machine vision and locates objects in satellite. Segmentation can be categorized as supervised or unsupervised learning/classification.

Automated image segmentation for cell analysis is generally a difficult problem due to the large variability (different microscopes, stains, cell types, cell densities, see Figure 2) and complexity of the data (possibly time-lapse images, acquired at multiple wavelengths, using multiple microscopes, and containing large numbers of cells). Nevertheless, screening the literature published on the subject since 1960, we find that the vast majority of cell segmentation methods are based on only a few basic approaches. Here we highlight the most common ones. Cell images may vary widely, depending on the type of microscopy and staining used, as well as the cell type and cell density. This makes the development of a generally applicable cell segmentation method a huge challenge [5].

Segmentation can be categorized as supervised or unsupervised learning/classification. The main requirement to validate a segmentation output is to know the 'ground truth', namely the true size, shape or other spatial features of object interest. The ultimate goal of blood cell segmentation is to extract blood cells from a complicated background and to segment every cell into morphological components such as nucleus, cytoplasm, holes and other organelles [3].

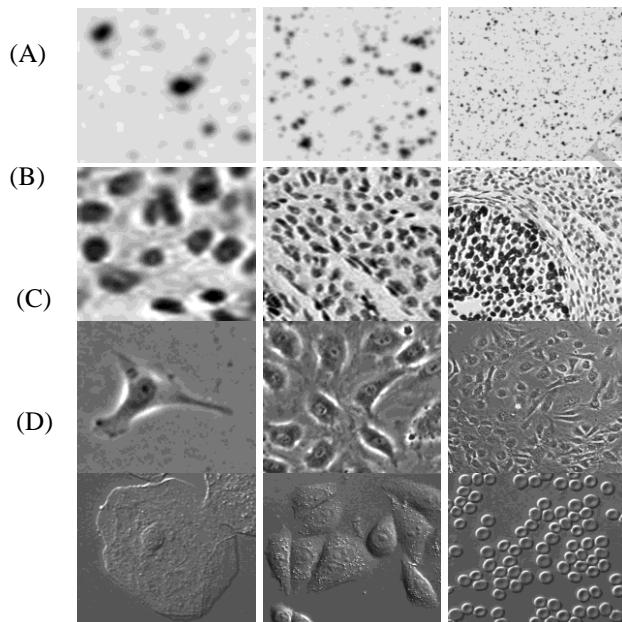


Figure 2 cell images (with cell density increasing from left to right),

acquired using bright-field microscopy (A-B), phase-contrast microscopy(C), differential interference contrast microscopy (D)

#### A. Intensity Thresholding

The first and, ironically, still one of the most predominant approaches to cell segmentation is intensity thresholding. The underlying assumption is that cells have significantly and consistently different intensities than the background (Figure 3A), globally, in which case a fixed threshold would suffice,

or locally, which requires adaptive thresholding. Approaches to automated threshold selection are usually based on statistical analysis of the global or local image intensities using the histogram. In practice, however, the fundamental assumption is often violated, and thresholding alone produces poor segmentation results. If at all, most cell segmentation methods apply thresholding only as a first step in the pipeline [5]. Thresholding was one of the earliest methods implemented for image segmentation. The simplicity of implementation and its intuitive properties gave image thresholding a central position in applications of image processing. In the case of cell segmentation, thresholding was followed by morphological operations in most of the cases. Thresholding is computationally cheap and fast.

#### B. Histogram-Based Techniques

The image is assumed to be composed of a number of constant intensity objects in a well-separated background. The image histogram is usually considered as being the sample probability density function of a Gaussian mixture and, thus, the segmentation problem is reformulated as one of parameter estimation followed by pixel classification. However, these methods work well only under very strict conditions, such as small noise variance or few and nearly equal size regions. Another problem is the determination of the number of classes, which is usually assumed to be known. Better results have been obtained by the application of spatial smoothness constraints.

#### C. Edge-Based Techniques

The image edges are detected and then grouped (linked) into contours/surfaces that represent the boundaries of image objects. Most techniques use a differentiation filter in order to approximate the first order image gradient or the image Laplacian. Then, candidate edges are extracted by the gradient threshold or Laplacian magnitude. During the edge grouping stage, the detected edge pixels are grouped in order to form continuous, one-pixel wide contours as expected. A very successful method was proposed by Canny according to which the image is first convolved by the Gaussian derivatives, the candidate edge pixels are isolated by the method of non-maximum suppression and then they are grouped by hysteresis thresholding. The method has been accelerated by the use of recursive filtering and extended successfully to 3D images. However, the edge grouping process presents serious difficulties in producing connected, one-pixel wide contours/surfaces.

#### D. Region-Based Techniques

The goal is the detection of regions (connected sets of pixels) that satisfy certain predefined homogeneity criteria. In region-growing or merging techniques, the input image is first tessellated into a set of homogeneous primitive regions. Then, using an iterative merging process, similar neighbouring regions are merged according to a certain decision rule. In splitting techniques, the entire image is initially considered as one rectangular region. In each step, each heterogeneous image region of the image is divided into four rectangular segments and the process is terminated when all regions are homogeneous. In split-and-merge techniques, after the

splitting stage a merging process is applied for unifying the resulting similar neighbouring regions. However, the splitting technique tends to produce boundaries consisting of long horizontal and vertical segments (i.e., distorted boundaries). The heart of the above techniques is the region homogeneity test, usually formulated as a hypothesis testing problem.

#### E. Markov Random Field-Based Techniques

The true image is assumed to be a realization of a Markov or Gibbs random field with a distribution that captures the spatial context of the scene. Given the prior distribution of the true image and the observed noisy one, the segmentation problem is formulated as an optimization problem. The commonly used estimation principles are maximum a posteriori (MAP) estimation, maximization of the marginal probabilities (ICM) and maximization of the posterior marginals. However, these methods require fairly accurate knowledge of the prior true image distribution and most of them are quite computationally expensive.

#### F. Hybrid Techniques

The aim here is offering an improved solution to the segmentation problem by combining techniques of the previous categories. Most of them are based on the integration of edge- and region-based methods. In, the image is initially partitioned into regions using surface curvature sign and, then, a variable-order surface fitting iterative region merging process is initiated. In, the image is initially segmented using the region-based split-and-merge technique and, then, the detected contours are refined using edge information. In, an initial image partition is obtained by detecting ridges and troughs in the gradient magnitude image through maximum gradient paths connecting singular points. Then, region merging is applied through the elimination of ridges and troughs via similarity/dissimilarity measures.

#### G. Fuzzy Based Techniques

Fuzzy methods have gained sufficient significance in recent times and are now used in major image segmentation techniques. In, fuzzy technique was used with the aim to allow a good processing of both vagueness and in determination characteristics of images, and the analysis of monochrome instead of colour images[6].

#### H. Colour Image Segmentation

R. Sukesh Kumar et al. [7] discussed about an approach for colour image segmentation using higher order entropy as a textural feature for determination of thresholds over a two dimensional image histogram. Two basic models for colour images are the RGB (Red, Green, Blue) colour model and the HIS (Hue, intensity, saturation) colour model. Two methods of colour image segmentation used RGB space as the standard processing space. These techniques might be used in blood cell image segmentation. Colour images are very rich source of information, because they provide a better description of a scene as compared to gray scale images. Hence, colour segmentation becomes a very important issue.

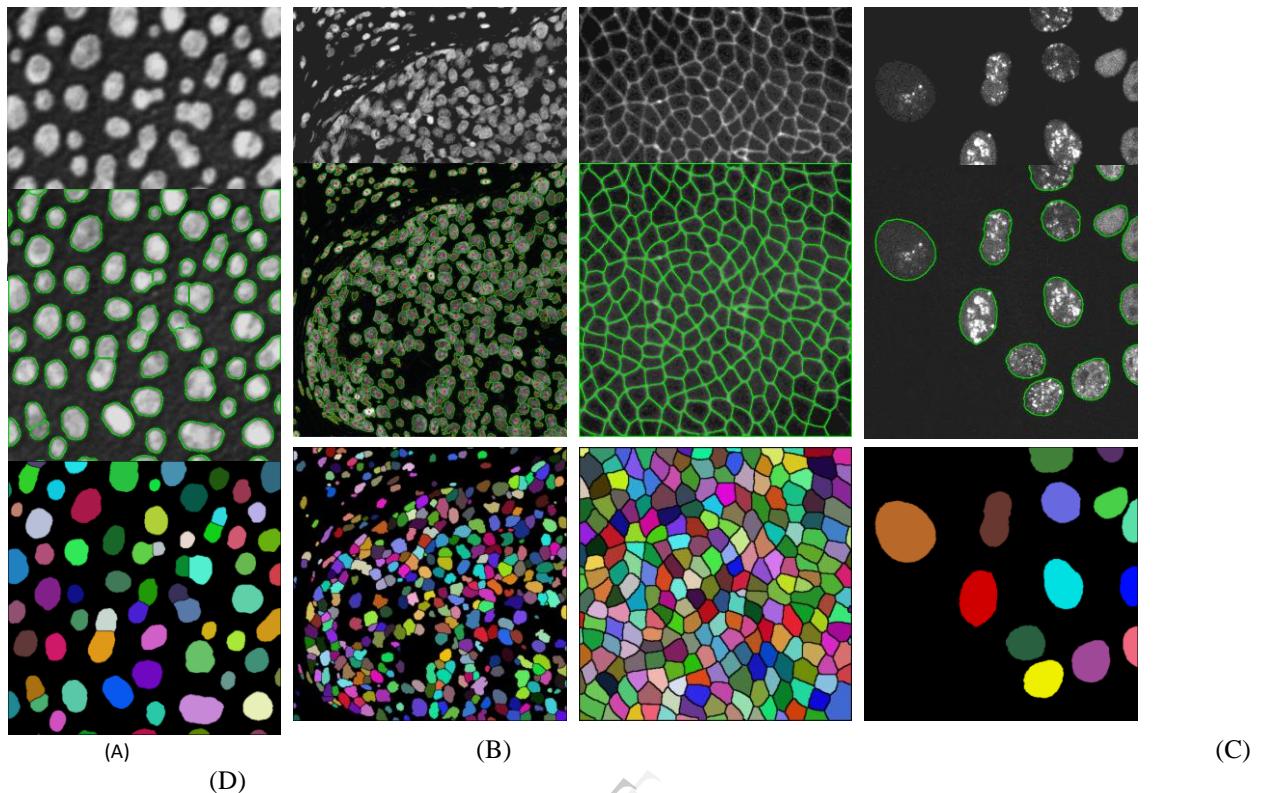
Colour image segmentation in HIS space requires conversion from RGB space to HIS space since maximum digital colour images are available in RGB format readily for segmentation in RGB space. Two algorithms have been proposed which are Non-Exclusive R, G, B segmentation and Exclusive R, G, B segmentation. Segmentation of an image into more regions would mean that there are multiple thresholds for the image. Region would be defined as a set of points having intensity values between two consecutive thresholds. This concept of multilevel thresholding has been implemented as an extension of entropy which would yield a set of thresholds instead of the conditional maximum giving a single threshold. The above algorithm has been applied to gray scale and colour images where the regions obtained are filled with different shades depending on the thresholds [3].

#### I. Watershed Transform

The watershed transform can be classified as a region-based segmentation approach. The intuitive idea underlying this method comes from geography: it is that of a landscape or topographic relief which is flooded by water, watersheds being the divide lines of the domains of attraction of rain falling over the region. An alternative approach is to imagine the landscape being immersed in a lake, with holes pierced in local minima. Basins (also called 'catchment basins') will fill up with water starting at these local minima, and, at points where water coming from different basins would meet, dams are built. When the water level has reached the highest peak in the landscape, the process is stopped. As a result, the landscape is partitioned into regions or basins separated by dams, called watershed lines or simply watersheds.

The advantages of watershed segmentation are threefold. Firstly, the results are connected regions with enclosed boundaries of single pixel wide, different from the traditional edge-based approaches generating disconnected contours. Secondly, the region contours adhere well to the real object boundaries. Furthermore, the combination of regions produced by watershed segmentation is equal to the entire image. Whereas, it is clear that some important drawbacks also exist. Watershed segmentation is sensitive to noise, the main reason leading to over-segmentation shown in figure 8. Besides, it doesn't work well in detection of thin structures and significant areas with low contrast boundaries.

As already stated, watershed segmentation suffers from the problem of over segmentation. Markers based segmentation servers as an ideal solution to this problem. A marker is connected component belonging to an image. Selection of markers comprises of two steps. First the image is pre-processed and then a set a criterion bust be defined to select a marker.

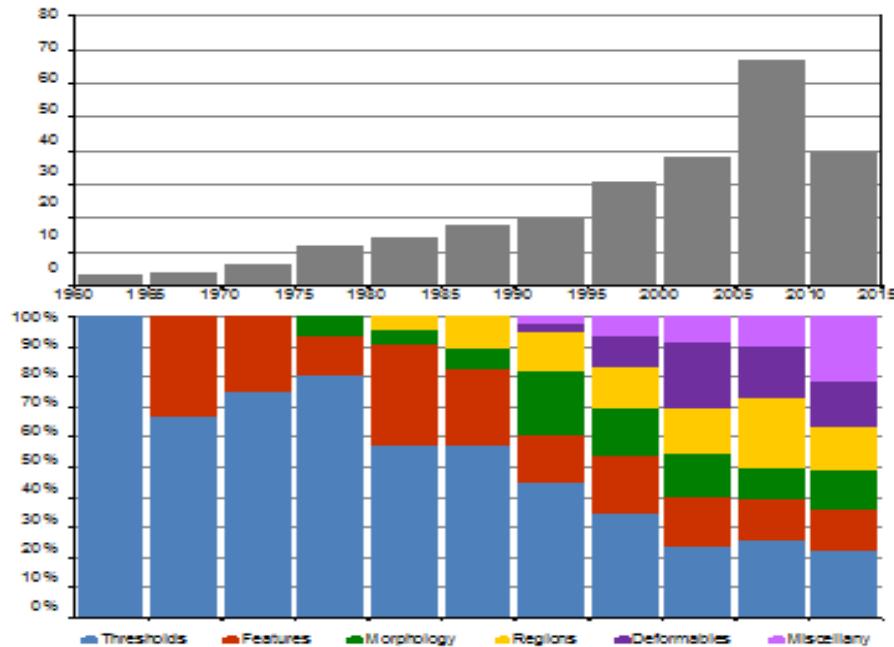


**Figure 3 Examples of cell image segmentation based on the discussed approaches.** The rows show, respectively, the input images, the automatically found cell contours (overlaid in green), and the corresponding labeled cell regions (arbitrary colors). (A) Cells that are fairly well separated and clearly brighter than the background are easily segmented using thresholding. (B) Scenarios with higher cell densities and intensity variations require more sophisticated methods. The method used here involves graph-cuts based binarization, Laplacian-of-Gaussian based cell detection (see red dots), and marker based clustering (C) Membrane stained images are ideally suited for watershed based segmentation.. (D) Studies of intracellular dynamic processes often result in images with significant intensity variations (in both space and time) and require robust cell segmentation and tracking methods. The method used here is based on level sets.

### III. CELL SEGMENTATION APPROACHES ANALYZED

It is interesting to analyze the usage of the different approaches to cell segmentation over the years shown in fig.4. Intensity thresholding, being the conceptually simplest and computationally cheapest of all, was the first approach to be used since the 1960s. Soon after, it was realized that differential features (in particular edges) could also be computed relatively easily, and might provide useful information. The field of mathematical morphology started to develop around the same time, and its basic operators were first used in the late 1970s for refining the results of thresholding based cell segmentation. During that same

Decade, all mentioned region accumulation approaches were conceived, and first examples of their usage in the context of cell image analysis appeared around the mid-1980s. Finally, the first deformable model fitting approaches for image segmentation were introduced in the second half of the 1980s, and were first applied to cell images in the early 1990s. Since then, miscellaneous other segmentation approaches found their way into the field, with as yet limited usage. Examples include dynamic programming, graph cuts, active masks, support vector machines, tensor voting schemes, neural networks, Markov random fields, and other concepts.



**Figure 4 Literature on cell image analysis shows an exponentially increasing interest in cell segmentation and the emergence of new approaches for this purpose the top panel shows their time histogram using lustrum bins. The bottom panel shows the breakdown of published methods per lustrum into six main classes of approaches (explained in the main text): intensity thresholding (blue), feature detection (red), morphological filtering (green), region accumulation (yellow), deformable model fitting (violet), and miscellaneous approaches (magenta) that could not be classified as any of the former. Most methods use a combination of several approaches.**

Several observations follow from the analysis of the literature on cell segmentation in the past 50 years. First, most of the different approaches were originally developed for applications in other fields (computer vision, robotics, materials science, medical imaging), and were later adopted for cell segmentation. This is remarkable, given the unique and unparalleled challenges in cell image analysis, which should provoke the development of original ideas. Second, even though new approaches are introduced once in a while, they seem to never fully replace old ones. Apparently, while none of them alone produces satisfactory results, they all continue to be useful to some extent. Third, as a consequence, methods proposed in recent times are rarely based on a single new concept, but are often merely new combinations of the discussed approaches, tailored to a specific application. Rather than converging to a robust, unified solution, it thus seems that the field is diverging, and by now almost as many cell segmentation methods have been developed as there exist cell analysis problems [5].

#### IV. CONCLUSION

Since there is no universal approach for obtaining accurate image segmentations, almost all techniques combine the two main approaches: region based schemes and edge based schemes. This is why a classification based on the criterion used by each segmentation technique is almost impossible. Instead, a list of the most used methods and how they are usually combined to achieve good segmentation results is helpful for better use of existing method and for improving

their performance as well as for designing new ones. In this paper we roughly describe some representative studies in the field of microscopic image segmentation.

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