Capture Largest Included Circles: An Approach for Counting Red Blood Cells

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Abstract. Complete Blood Count (CBC) is a standard medical test that can help diagnose various conditions and diseases. Manual counting of blood cells is highly tedious and time consuming. However, new methods for counting blood cells are customary employing both electronic and computer-assisted techniques. Image segmentation is a classical task in most image processing applications which can be used to count blood cells in a microscopic image. In this research work, we have employed a few existing segmentation techniques, and also proposed a new scheme to count total blood cells in a smear microscopic image. The proposed technique, called Capture Largest Included Circles (CLIC), is a parameterized segmentation algorithm that captures largest possible circles in an object boundary. The algorithm is perfectly suited for appliance in counting blood cells because of high circularity ratio of cells. Comparative study of segmentation by CLIC and a few other state-of-the-art segmentation algorithms such as Distance Regularized Level Set Evolution (DRLSE), Watershed segmentation and Pixcavator (a topology-based segmentation) is also part of this research work**.** Results have proven the superiority of CLIC over other schemes, especially in case of diseased red blood cells.

Keywords: Red blood cells, Level set, Watershed, Pixcavator, CLIC.

1 Introduction

Red Blood Cell (RBC) counting is one of the most commonly performed medical tests as it plays vital role in the diagnosis of various diseases like Alport syndrome and Anemia[1]. Blood smear is examined under microscope and count of each type of cells is obtained. Counting process, when performed manually, proves to be tedious, time consuming and subjective. Results vary from person to person and even for one person at different turns. Precision of the process is also very low because of subjective evaluation by histopathologists. Moreover, factor of human error adds to the delicacy of the process. Therefore, some automatic and accurate mechanism is required to cope with the aforementioned problems. Automatic blood cells inspection may be performed with the help of automatic flow cytometry, but algorithms based on image processing and computer vision techniques reduce analysis cost. Therefore,

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researchers have been putting their efforts in this direction since last decade and have proposed various automatic, computer-aided blood cell counting techniques.

Counting of RBC is the overall task at hand. It comprises of several steps; out of which segmentation and counting of cells are the two most important phases. Our work is primarily focused on these two steps of automatic computer-aided RBC counting. We have applied traditional image segmentation approaches to blood cells microscopic images to count the number of cells but our main contribution is to present a new scheme for RBC counting, called CLIC. CLIC is a parameterized segmentation algorithm that captures largest possible circles in an object boundary in an efficient way. Results reveal that the proposed scheme outperforms classical image segmentation algorithms.

Rest of paper is organized as follows. Section2 investigates literature on automatic computer-aided segmentation and counting of RBC. Section3 reviews watershed, DRLSE and Pixcavator algorithms. Section4 presents in detail the CLIC approach. Section 5 elaborates the results of applying the said techniques on sample blood cells microscopic images. Section 6 supplies a performance overview whereas section7 concludes the paper.

2 Previous Work

Segmentation and counting of the RBC in a microscopic blood smear image is a potential research problem due to its medical significance. Researchers have been putting serious efforts for this purpose, and much encouraging and accurate results have been produced as a result of these endeavors. Following text states a brief survey of literature related to the computer vision based blood cells segmentation and counting.

Boray et al. [2] employed watershed transform in combination with Radon transform to obtain effective segmentation of peripheral microscopic blood images. Initially, they applied a special form of watershed transform, called minimum area watershed transform, to obtain initial segmentation. This initial segmentation served to locate markers in the image using circle Radon transform. The result of applying circle Radon transform is utilized subsequently in a marker controlled watershed transform to obtain final segmentation result. The results were evaluated on a benchmark set and a segmentation rate of 95% was achieved. Similarly, Ruberto et al. [3] claimed to propose a more accurate approach to segment blood images than the classical watershed algorithm. They used gray scale granulometries on blood images for this purpose. Gray scale granulometries were based on opening with non-flat disk-shaped structuring elements. The non-flat nature of structuring element helped to enhance the circular shape of blood cells as well as their compactness. Then, they have employed a flat disk-shaped structuring element to separate occluded cells. A similar approach to [3] was adopted by Dorini et. al. [4]. They also used morphological operators for segmentation of white blood cells in blood smear image. But, they improved the segmentation accuracy by incorporating the scale-space properties of a toggle operator. Similarly, an excellent work in the field of granulometry for blood

cell counting was performed by Theera et al [5]. Authors estimated blood cell counts against each category according to cell-age. They utilized non-homothetic theory for their purpose because each cell class is attributed to a random grain and non-homothetic theory is ideal for such a problem. After applying the approach, authors demonstrated low counting error for different cell categories.

Theerapattanakul et al. [6] utilized the benefits of snake/active contour to segment white blood cells in microscopic blood smear images. They started by thresholding the image to obtain a binary image that was used to compute the locations of nuclei of white blood cells. Then they cropped the smear image in the vicinity of found nuclei and applied the active contour method on each cell. They placed the round shaped snake at the center of the nuclei and the snake was allowed to expand. After sufficient iterations, the snake evolved to the shape of white blood cell hence segmenting the cell from the image. This process of cropping each cell with its neighborhood and evolving round shaped snake inside that cell was carried out for the whole image to segment all possible white blood cells in the image. In the end, authors demonstrated visual results of their approach and proved effectiveness of their approach.

Seongeun et al. [7] extracted Leukocytes (white blood cells) from blood smear images using region-based active contour approach. Regional statistics were used to avoid the problem associated with initialization of the contour. They were also utilized to attract the evolving contour towards the boundaries of white blood cells. To control the contour deformation near edges of Leukocytes, they used an additional regularizer. Specifically, the active contour model used in this approach was the level set method. The results of applying this method on an image database were compared with those of already categorized by expert hematologists. To be precise, nucleus of different types of cell images (Neutrophil, Lymphocyte, Monocyte and Eosinophil respectively) were segmented with an accuracy of 91.40, 95.45, 90.83, 88.43 percent and cytoplasms were segmented with an accuracy of 93.60, 95.10, 91.82, 91.41 and 93.02 percent respectively.

Acton proposed a new external force [8] in the active contour model for tracking Leukocytes from intra vital microscopic video imagery. The proposed force field, referred to as Motion Gradient Vector Flow (MGVF), takes hemodynamic direction of flow into consideration. This force adds to the classical Gradient Vector Flow (GVF) proposed by Xu and Prince [9] and it is realized in the active contour model by minimizing an energy functional which considers motion direction and image gradient magnitude into its formulation. The proposed active contour model employing the MGVF was evaluated against the classical GVF on intra vital microscopic video imagery. It was shown that root mean square error of tracking Leukocytes at different frame rates for MGVF was much less than GVF.

3 RBC Counting Techniques

Several methods have been proposed by researchers for RBC segmentation and counting with each having its own merits and demerits. In this study, we have used three traditional segmentation techniques for RBC counting.

3.1 Watershed Segmentation

Watershed scheme [10] is inspired by the watershed in hilly areas. It finds watershed lines that represent low intensity areas around high intensity objects. Core of watershed segmentation is internal and external markers. Internal markers are exploited to limit the number of regions by specifying the objects of interest; mainly they are used as seed points for region growing. However, external markers are best candidates for representing image background. Watershed, in a standalone position, can perform reasonably well however in order to make the segmentation process more effective, we used a sequence of morphological operations in pre-processing and post-processing phases.

3.2 Distance Regularized Level Set (DRLS) Evolution

Level set is a viable method for capturing dynamic interfaces using contour evolution. The key ideas of level set method were established by Dervieux and Thomasset [11], [12] but level sets did not attain significant attention before Osher and Sethian [13]. After its theoretical birth in 1988, level set method has found various applications in the fields of computational physics, fluid mechanics, computer graphics, image processing and computer vision. The evolution of level set has witnessed many flavors and variants. DRLSE [14] is one such variation that not only does away with the burden for re-initialization but also permits greater flexibility in the selection of initial Level Set Function (LSF). In our implementation, we have used a binary step function which is extremely efficient to generate. Also, a finite difference scheme is employed as proposed in [14].

3.3 Pixcavator

Pixcavator is one of latest segmentation techniques that have been developed for counting RBC. In pixcavator [15], Saveliev et al. extended the idea of [16] and exploited the topological relationship between different objects in the image to identify and count number of cells in the image. Though, scheme is developed only for RBC counting, yet it has proven to be simple and effective. It can be used in other scenarios with minor modifications.

Counting the number of blood cells after segmenting them in a microscopic blood smear image is simply a task of listing the number of connected components in the image. If the segmentation is performed accurately and the cells are separated after processing, then cells constitute different connected components in the image. In our work, we have applied three classical and one newly proposed technique. The output from these segmentation techniques is an image containing connected components in place of blood cells which can then be counted easily using a simple connected component counter algorithm.

4 Proposed RBC Counting Solution

Counting of red blood cells, though seems simple, is actually a challenging task. Several algorithms proposed in the past exhibit good performance for healthy cells but performance degrades gradually as the cells become diseased. We have proposed a very simple, straight forward yet effective algorithm, **CLIC** for counting red blood cells. We have followed the idea of [17, 18] and modified their algorithm to meet the specific needs of the problem at hand. In [17], initially they found 4-connectivity based connected components from the image. Then, they fitted all possible circles in the connected components by iteratively processing individual components. However, they slightly varied the algorithm in [18]. In the first step, they made connected components and found only one largest possible circle from each component. In successive iterations, they found remaining pixels that are not part of any circles, made connected components of those pixels and fitted only one largest possible circle in each component. Process continued until all the remaining components have size smaller than an area threshold. Both the schemes perform equally well in terms of segmentation accuracy but second scheme works very efficiently as far as memory utilization and CPU processing is concerned.

In our work, we have combined both the algorithms and simplified them. Our algorithm has proven to be computationally faster because instead of working on connected components, we have processed whole image at once. Several other variations that we have introduced in our algorithm are pre-processing, and customized circle growing in post-processing phase.

4.1 Capture Largest Included Circle Algorithm

Sequential steps of CLIC are:

Step1. Read the input image.
Pre-Processing
Step2. Convert the image to binary if it is colored or grayscale.
Step3. Perform morphological operation of dilation and hole filling.
Step4. Remove areas of image smaller than an area threshold.
Step5. Perform rigorous erosioning.
Algorithm
Step6. Specify maximum and minimum possible radius threshold.
Step7. Generate simulated circle of given radius (largest).
Step8. Iterate the circle on the entire image and match where, in the image, such pattern exists.
Step9. In case pattern found and the underlying image pixels are not already part of some other circle, a circle exists at that place.
Step10. Mark the remaining pixels that are not part of any circle.
Step11. Decrement the radius value and continue from step7 (use unprocessed pixels in each next iteration).
Post-Processing
Step12. Approximate exact appearance of cells.

It is worth to be noted that in step 08 of the algorithm, a simulated circle of given radius is treated as a pattern, comprising of pixels. Pattern is overlapped at each image pixel and it is checked whether the underlying pattern is similar to circle or not.

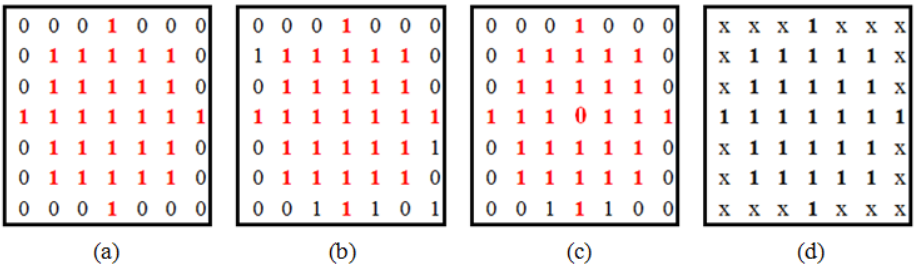

Fig. 1. (a) Simulated circle, (b) & (c) sample image portions, (d) points of matching

In circle matching, only those pixels of circle and underlying image are matched where '1' lies in circle pattern. Matching does not care rest of the pixels of the image and circle. This phenomenon is depicted in Fig. 1(d). In Fig1, point of contact of image and circle is row number 4 and column number 4 of the given samples. Algorithm will detect circle (red pixels) in Fig. 1(b) by ignoring the '1's outside circle boundary whereas it will not detect circle in Fig. 1(c) because of middle zero.

In step 12 of the algorithm, once circles are located at different places in the image, then in order to approximate the exact appearance of underlying red blood cells, a customized connected component analysis is performed. Here, we start from the detected circle boundary and move outside. In each outer layer, we add a pixel to the circular object if three pixels in the inner layer bear 8-connectivity to the pixel and no other 3 pixels that are not part of current circular object, share 8-connectivity to this pixel. Although, a circle may penetrate in another circle but that happens very rare because erosioning have already defined boundaries between objects.

4.2 Circle Generation and Connected Components Removal

Component removal and circle generation algorithms are further elaborated in Fig. 2. In connected components removal, components with size smaller than an area threshold are removed because of obvious reason that they are smaller enough to contain circles. Removal of connected components is based on a pre-defined area threshold; area is actually number of pixels of connected component. This step greatly simplifies the algorithm as number of connected components, generated because of noise, reduces to a great extant.

In circle generation step, circles of different radii are generated in successive iterations. A rectangular array having rows and columns, equal to two times the given radius, is spawned. Center of rectangle is treated as center of circle and for each pixel, it is checked whether it lies inside or outside circle boundary. Array indices are marked accordingly; 1 for pixels inside and on boundary and 0 for pixels outside boundary. In circle generation, maximum and minimum radii were chosen to be 15 and 8 respectively. These radii were selected after a rigorous analysis of several images of red blood cells.

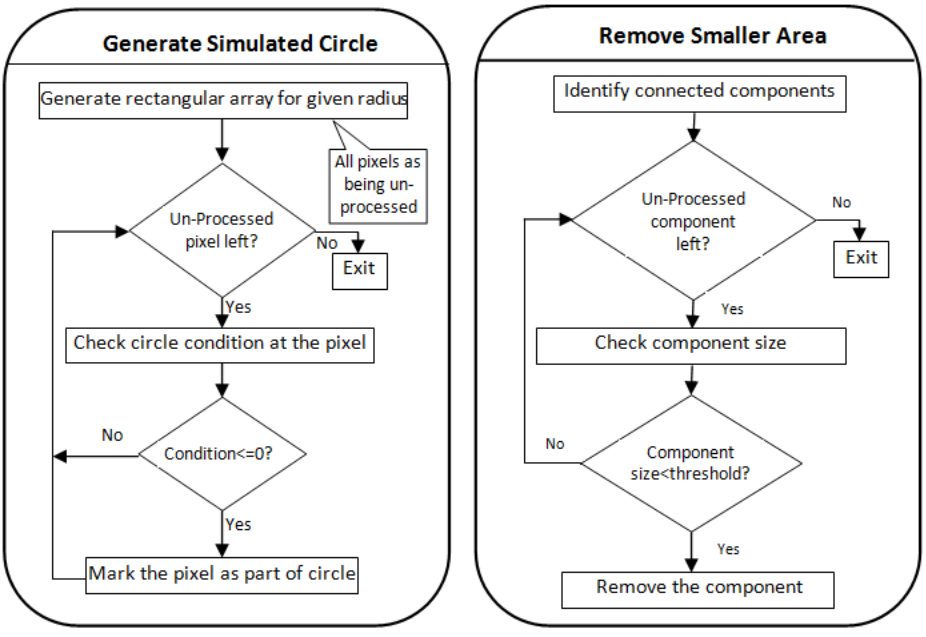

Fig. 2. Circle Generation and Connected Component Removal Algorithm

5 Experimental Results

We carried out experiments on a set 15 images out of which 8 images were healthy and 7 were diseased. Segmentation was performed by using watershed, level set, Pixcavator and our scheme. Several configurable parameters of the algorithms are dumped in Table. 1 whereas Fig. 3 provides a pictorial view of segmentation results.

Table 1. Configurable Parameters of Segmentation Algorithms

Parameter Name = Value (Range of Values)	
Watershed	**Pixcavator**
Type of structuring element = disk Radius of disk = 8 (preprocessing for extracting markers)	shrink factor = 1
Level Set	**CLIC**
time step = 4, lambda = 5 mu = 0.2/time step, epsilon = sigma = 1.5 alpha = -5(healthy),-2(diseased)	component size threshold = 50 min radius = 08 max radius = 15

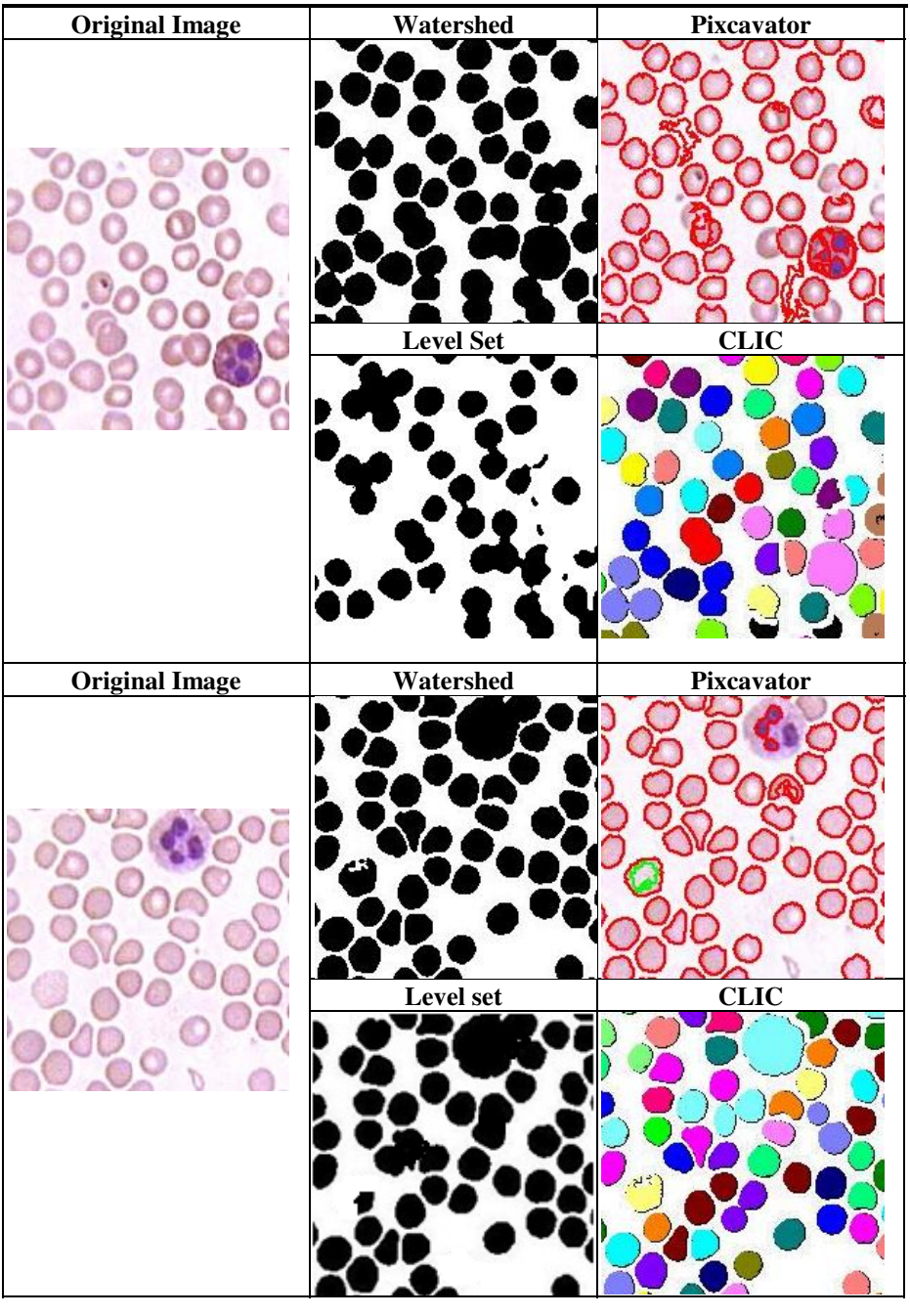

Fig. 3. Results of Segmentation Algorithms

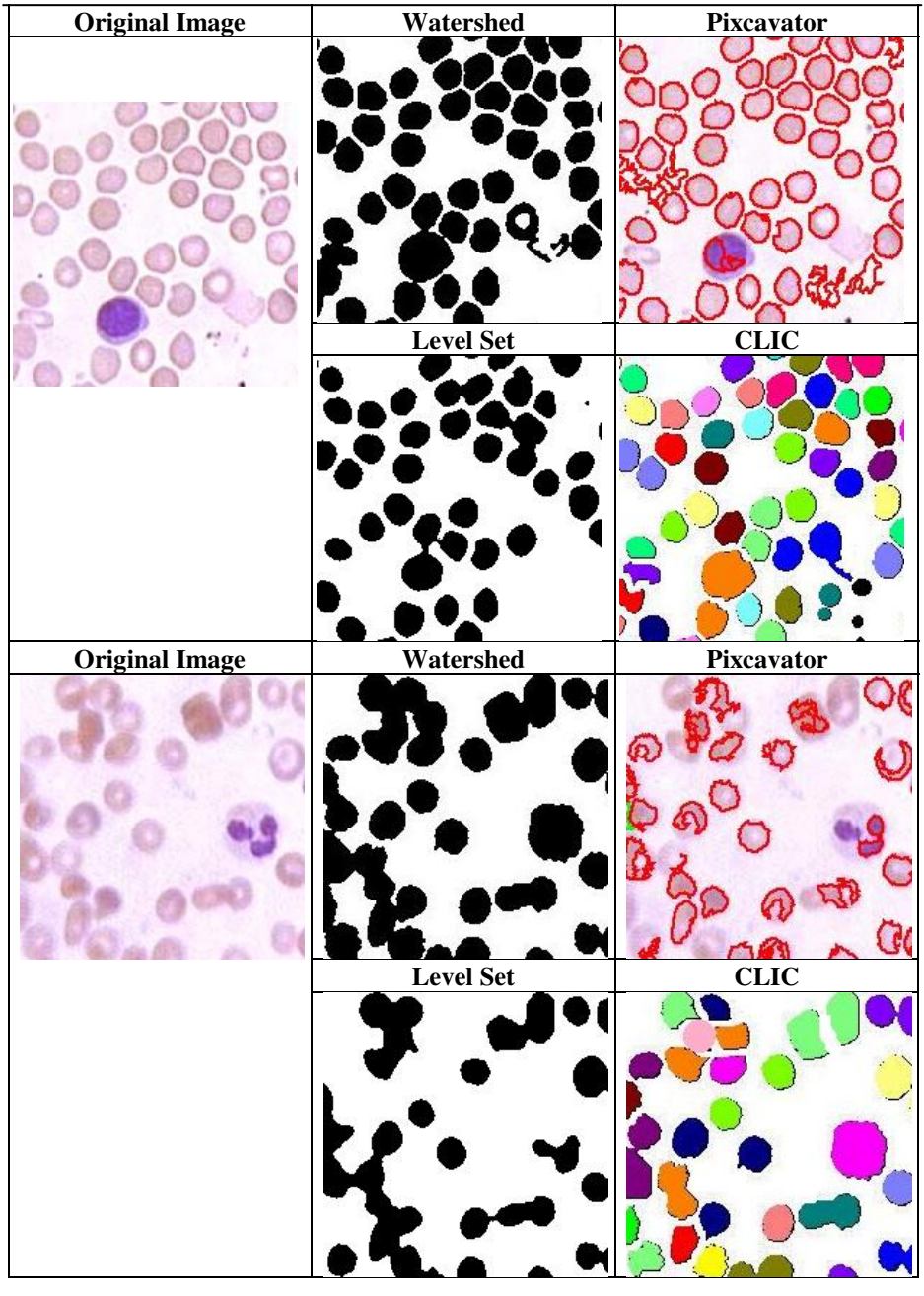

Fig. 3. (*continued*)

Segmentation results, shown here, are not the entire RBC images. In order to provide a clearer and enlarged view of segmentation, same portion has been cropped from the respective images. Original resolution was 400x670 but we have cropped 100x100 sections from the images starting from 100^{th} row and 100^{th} column. However, Table2. lists total number of cells detected in images. Second column depicts image category whereas third column lists total cells counted using manual segmentation. Remaining columns show segmentation results for individual algorithms.

6 Discussion

Segmentation results lead to a set of very important conclusions. Counting of RBC from diseased image is more error-prone as compared to counting from a healthy image. This effect is painted in Table2. where we see that segmentation results for healthy images are much superior to that of diseased images regardless of the choice of segmentation algorithms. As far as individual algorithms are concerned, level set faces major challenges in segmentation of both the healthy and diseased images; weak edges of cells prevent contour from penetrating through the middle regions of the image. Hence level set becomes unable to capture smaller details in the image. Watershed and Pixcavator both share a comparable level of performance in case of healthy images but the performance of watershed degrades drastically in case of diseased images, where it detects only half of the circles as detected by Pixcavator. Watershed, though segments the image to reasonable level, does not respects natural boundaries between different cells hence it treats multiple individual cells as one merged component.

Table 2. Total cells segmented using individual algorithms

Image	Category	Total	Watershed	Pixcavator	DRLSE	CLIC
1	Healthy	415	269	322	167	326
2	Healthy	398	346	341	124	371
3	Healthy	370	318	334	197	352
4	Healthy	406	352	346	150	368
5	Healthy	457	401	378	167	393
6	Healthy	356	297	308	106	310
7	Healthy	395	321	345	147	361
8	Healthy	342	258	286	158	311
9	Diseased	243	80	148	71	160
10	Diseased	292	100	180	89	210
11	Diseased	252	110	154	78	198
12	Diseased	281	90	165	102	238
13	Diseased	192	84	134	67	169
14	Diseased	234	108	157	98	158
15	Diseased	247	123	198	105	201

Our algorithm performs much better in both the cases. Though, there is only a slight improvement in case of healthy images but our algorithm outclasses other segmentation algorithms for diseased cells. From Fig. 3, it is evident that cells that have either not been detected or have not been separated by other segmentation schemes, have been correctly detected and separated in CLIC. Fig. 4 clearly depicts the best percentage accuracy of CLIC both for healthy and diseased cells.

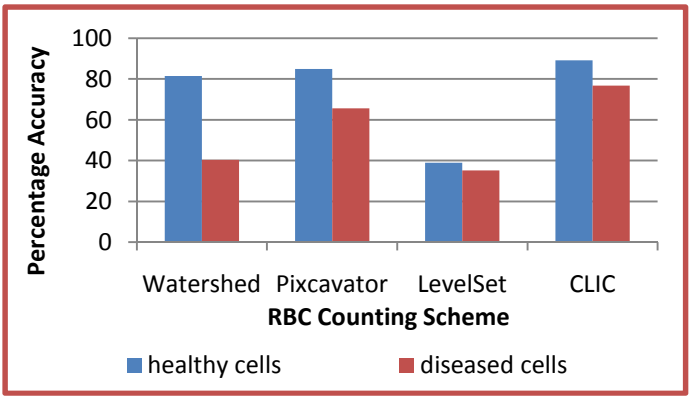

Fig. 4. Percentage Accuracy of segmentation techniques for healthy and diseases cells

7 Conclusion and Future Work

Counting the number of blood cells in a blood smear image is a standard medical test that plays vital role in diagnosis of various diseases. Several computer vision techniques exist for fast, accurate and cost effective counting of these blood cells. Segmentation is the major task in computer vision domain through which a total count of blood cells is obtained. In this work, we have applied several segmentation techniques to segment and subsequently count the number of blood cells. Our main contribution is to present a new technique for RBC counting and compare its results with other classical segmentation approaches like watershed and level set. Results have proven the superiority of our proposed scheme especially in case of diseased images.

Though segmentation results are quite promising, CLIC is still a naive idea in the field of RBC counting. It can further be fine-tuned to produce more robust results. The circle size is currently fixed and is specified as parameter to the CLIC algorithm. In future, an adaptive circle size may be introduced that may increase the accuracy and effectiveness of CLIC. Also, several other performance parameters and state-of-the-art segmentation algorithms may be added to provide better comparative study.

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