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Applied Medical Informatics in the Detection and Counting of Erythrocytes and Leukocytes through an Image Segmentation Algorithm

Ana Carolina Borges Monteiro, Yuzo Iano, Reinaldo Padilha França and Rangel Arthur

Abstract— **More than half of the medical decisions rely on laboratory tests, which are essential to complete diagnosis or to refer the patient to more specific tests. In this context, the blood count is the most requested laboratory test. Even though there is a wide variety of CBC equipment, many are similar in cost. The automated methodologies present high speed and high accuracy. However, the high cost is often incompatible with the cost of living of people living in less-favored countries. In this way, it is essential to develop methodologies that reduce the cost of the blood count. The present study builds on the development of a laboratory medical algorithm for the detection and counting of erythrocytes and leukocytes in digital blood smear images. The algorithm employs the Hough Transform and the detection of objects by coloration. The deployment and performance analysis of the algorithm were performed in the virtual environment of Matlab software. The experiments were conducted through 10 digital images from open-access platforms with later analysis of sample execution times through the "tic toc" function. The results of the quantifications were expressed separately. The methodology developed showed high accuracy (90%) as well as low time to execute each of the images analyzed, with the average execution time being less than 2 seconds. Therefore, this study can be considered the first step in the accomplishment of hemograms with low cost, greater accessibility, and speed without the loss of reliability of the method.**

Index Terms— **Hematology; Blood cells; Erythrocyte indices; Biomedical engineering; Medical informatics; Cytology; Hough transform.**

I. Introduction

The hemogram is a highly requested laboratory test in the medical routine because it directly provides the diagnosis of pathologies and pinpoints several diseases. This test consists of the erythrogram, leukogram, and platelet, which quantitatively and qualitatively evaluate erythrocytes, leukocytes, and platelets, respectively [1].

The hemograms' automation (HA) expedites exams and their reports. However, they are more expensive than manual exams. HA began in the 1950s when Coulter Eletronic, Inc. introduced the impedance principle for cell counts. Later, in the 1960s the conductivity technique based on the high-frequency electromagnetic current provided information about cell volume, nucleus size and cytoplasmic content of granulations [4].

Then, in 1970, laser beam scattering and hydrodynamic focus techniques were introduced. Both techniques preserve nuclei and granulocytes of leukocytes, retracting only the cytoplasmic membrane. These techniques exploit the principles of diffraction, refraction, and reflection of the light emitted. However, in these techniques, the red blood cells are undetectable. In order to solve this problem, the red cells started to be counted utilizing flow cytometry and hydrodynamic focus, where these cells are counted one by one through an extremely fine capillary [4].

Currently, there are a large number of multiparameter devices, which use the impedance, conductivity, and dispersion techniques of emitted light. However, before acquiring a hematological device it is necessary to consider the following parameters: automation device x type of patient attended; the number of hemograms day x samples/hour of the apparatus; cost of each blood count; quality control; technical assistance; and staff training [3] [4].

However, even with the acquisition of hematological equipment, the manual hemogram is not a dispensed practice and is recommended for the confirmation of hematologic reports of pediatric patients, patients over 75 years of age, cancer patients, patients with suspected leukemia or polycythemia, patients with leukocytosis and patients in severe condition [4].

In recent years, techniques developed in the engineering field have demonstrated the potential to solve problems and/or innovations in the medical areas in order to benefit both medical professionals and patients.

In the year 1970, was created Matlab software. While other computational languages work mainly as numbers one at a time, Matlab can operate on all matrices and commands. All the variables of this tool are multidirectional commands, regardless of data type [5] [6]. Matlab applicability in digital image processing [16] result from an extensive set of multidimensional arrangement processing functions

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of image arrays. The Matlab IDE eases image-processing operations in a compact and clear way being ideal for solving image processing problems [7] [8].

Ji Y. Xie, introduced the Random Hough Transform (HT) in the detection of red blood cells to improve the detection curve of precision and robustness, as well as computational efficiency [9]. Subsequently, Smereka modified the HT, proposing improvement in the detection of low-contrast circular objects [10]. Already, Arivu and colleagues performed the counts of red cells and leukocytes by differentiating the distinct morphological characteristics that these cells present with each other [11]. Humaimi carried out research whose objective was to develop a computer vision system capable of detecting and estimating the number of red blood cells and leukocytes in an image [12]. Recently, Sahastrabuddhe et al. carried out a study with 5 patients, where digital images of red cells and leukocytes were present in blood smears, and the segmentation of the image was performed through the Hough Circular Transform [13].

Thus, in this context, it is well known that the HT is robust, valuable, and used in many fields of science, in the same way, that it is a fine-grained segmentation of radio or TV broadcasts [20 - 28]. The use of digital images has shown explosive growth in the last decades, where it is considered impossible to list so many modern applications that involve digital images, mentioning among them areas of special relevance as broadcasting [20 - 28].

Techniques for medical diagnostic imaging are an increasingly present trend fueled by the daily routine of medical activity, which is marked by a constant search for an increasingly accurate diagnosis [34 - 41].

With this focus on this horizon, this paper aims to develop an image segmentation algorithm that is capable of detecting and counting erythrocytes and leukocytes present in digital images of blood smear fields stained, with the purpose of developing a low-cost methodology, without the need of specific equipment and that is accessible disadvantaged populations.

II. Methodology

The research was developed at the Faculty of Electrical Engineering and Computing (FEEC) of the State University of Campinas - UNICAMP, Campinas, Brazil. The experiments were conducted through the Matlab software, where an algorithm was developed based on the union of the HT methodologies and the detection of objects by coloration. Thus, the algorithm was named HT-DC, an acronym that originated "Hough Transform and Detection Color".

The HT was introduced by P.V.C. Hough in 1962, in order to detect the lines and arcs in the photographs obtained in chambers of clouds. The HT is classified in the middle range of the image processing hierarchy. This methodology assigns a logical label to an object that existed only as a collection of pixels. Therefore, it can be classified as a segmentation procedure [14] [15].

The idea behind the method is simple: parametric shapes in an image are detected through points accumulated in the parameter space. As a result, if a particular shape is present in the image, the mapping of all its points in the parameter space must be grouped around the parameter values that correspond to that shape. This approach maps distributed and disjoint elements of the image to a localized accumulation point [14] [15]. The logic of the creation of the HT-DC algorithm and the stages of the detection and counting process of red blood cells and leukocytes are presented in Figure 1:

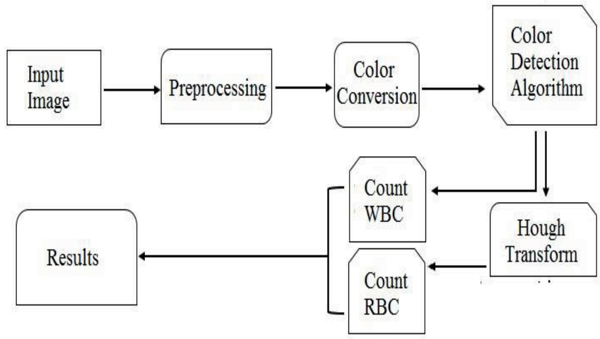

Figure 1 - Logic of the Hough Transform and Detection Color (HT-DC) algorithm for the detection and counting of erythrocytes and leukocytes present in digital blood smear images.

The HT-DC methodology is dependent on digital images, which is defined as a two-dimensional function *f (x, y),* where *x* and *y* are spatial coordinates, and the amplitude *f* for any pair of coordinates is called image intensity. The term grayscale is often used to refer to the intensity of monochrome images. The intensity of the level of each pixel is essential information about the image. Pixel intensity values are also used to perform operations such as segmentation or filtering. In addition, one can use the intensity to extract information, such as the number of cells in an image [5] [17]. In this context, colored images are formed by combining individual images. In the RGB (Red, Green, and Blue) color system a color image consists of 3 individual monochrome images referred to as primary images or red, green, and blue components [7] [18].

The experiments were conducted through images obtained through hematology database open access: https://imagebank.hematology.org/. These images of microscopic fields have images of erythrocytes and leukocytes in the non-pathological state. These images were acquired in digital format 'png', 'jpg' or 'jpeg' and were later transferred to Matlab software. Subsequently, the image is subjected to the pre-processing and color conversion phase in order to correct problems that may arise during the capture of the images under an optical microscope, such as brightness, contrast, and sharpness. The image is then subjected to the color detection algorithm. This tool aims at detecting and counting leukocytes, which are detected and counted through their azurophilic staining. Finally, the results of erythrocyte and leukocyte counts are released separately.

Detection by staining avoids erroneous counts of blood cells since erythrocytes and leukocytes are circular and can easily be counted without distinction by the HT. Thus, the blue staining of the leukocyte nuclei can be easily separated from the image by means of the RGB staining separation process, preventing leukocytes from being counted as erythrocytes.

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As discussed earlier, a digital image is defined through the *x, y* coordinates. In this way, the detection algorithm by coloring marks the leukocytes as the "+" sign, which indicates the center of each detected form. The detection of objects by staining can be done through the Matlab Image Processing Toolbox.

Due to the morphological characteristic of the red cells having a biconcave disk format, the HT is applied in order to detect circular objects in the images. The HT is responsible for detecting erythrocytes present in the digital image. For this, the green color component was extracted from the image by the HT-DC algorithm, since it contains the maximum value necessary for this type of segmentation. It is possible to develop logics from the HT, how to draw circles around the detected cells. The HT-DC algorithm detects the edge points in each circle and draws the best possible circle around each cell. This developed methodology also uses a 3-D matrix, the first two dimensions being responsible for representing the coordinates of the matrix, which increases each time the circle is drawn around the rays on each edge point. An accumulator is responsible for maintaining proper counting [31, 32, 33].

Subsequently, the HT-DC algorithm was evaluated for its accuracy and execution time. The accuracy was determined by the values obtained in the manual counts performed by a biomedical professional with a comparison with the values found in the counts of red cells and leukocytes emitted by the HT-DC algorithm. This process uses 10 images of optical microscopy fields stained from free open-access platforms were used to avoid the need for submission and approval of the project by the Research Ethics Committee (CEP).

For the analysis of the execution time of the HT-DC algorithm, the "tic toc" function was used via the command line at the Matlab software prompt, and it was responsible for measuring the execution time of each of the analyzed samples, that is, this function quantifies the time that the image needs to perform a simulation [6] [7]. The "tic" function starts a timer to measure performance, recording the internal time of the "tic" command execution - and displaying the elapsed time with the "toc" function [6] [7].

In addition to detecting and counting erythrocytes and leukocytes, the HT-DC algorithm has a function capable of determining the erythrocyte indices. For this, the algorithm is based on the mathematical formulas previously determined by the literature. The determination of the Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin (HCM) and Mean Corpuscular Hemoglobin Concentration (CHCM) are dependent on hemoglobin, hematocrit, and total erythrocyte counts, as presented in Equations 1, 2 and 3 [1] [2] [3] [4].

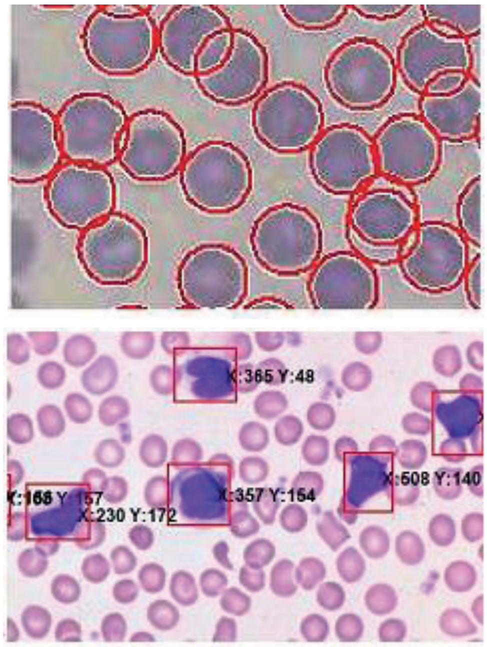
$$CVM = \frac{Hematocrit\ x\ 10}{Erythrocytes\ Count} \quad (1)$$

$$CHM = \frac{Hemoglobin\ x\ 10}{Erythrocytes\ Count} \quad (2)$$

$$CMCH = \frac{Hemoglobi\ \ 100}{Hematocrit} \quad (3)$$

III. RESULTS

The detection and counting of erythrocytes occur by inserting a circle around each of the red cells present in the image of the blood smear field, while the leukocytes are marked by a "+" at its center and are accompanied by your description of coordinates in the image. The way the HT-DC algorithm detects and counts red blood cells and leukocytes is shown in Figure 2.



(a)

(b)

Figure 2 - Hough Transform used in the detection of red cells (a) with detection of leukocytes by staining (b)

Microscopy fields' images with erythrocytes and leukocytes underwent manual counts by a biomedical professional. Afterward, these images were transferred to the HT-DC image segmentation algorithm, where red blood cells and leukocytes were detected and counted with the values released separately.

Then, the erythrocyte and leukocyte values obtained by means of the manual counts and the counts by the HT-DC algorithm were compared to each other, as shown in Figures 3 and 4. Through the values shown, it is possible to note that the accuracy of the erythrocyte and leukocyte counts is 90%.

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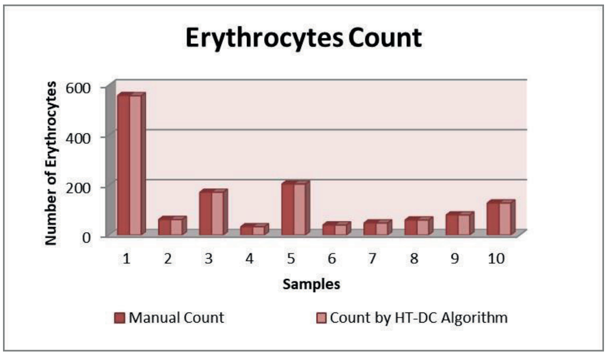

Figure 3 - Comparison of erythrocyte counts by manual methodology and HT-DC algorithm

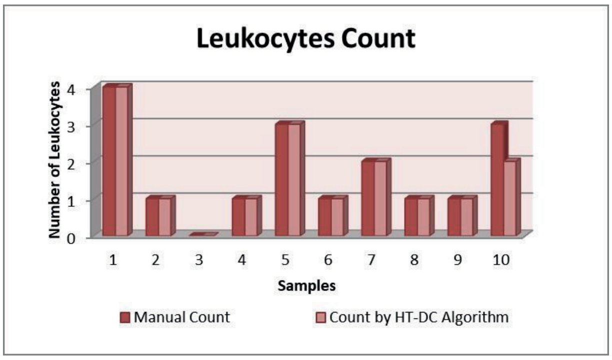

Figure 4 - Comparison of leukocytes counts by manual methodology and HT-DC algorithm

The detection and counts of erythrocytes and leukocytes by the HT-DC algorithm were performed in computers with different configurations: (1) Intel Core i3 processor, with 4GB RAM and (2) Intel Core i5 processor with 8 GB RAM. The run-time results of the samples are shown in Figure 5 and 6:

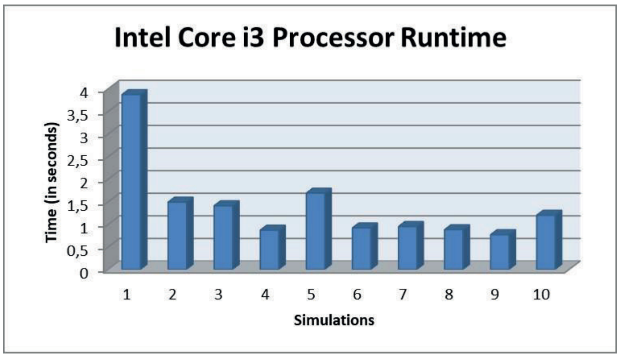

Figure 5: Time of execution of the samples submitted to the Hough Transform and Detection Color (HT-DC) algorithm on an Intel Core i3 processor

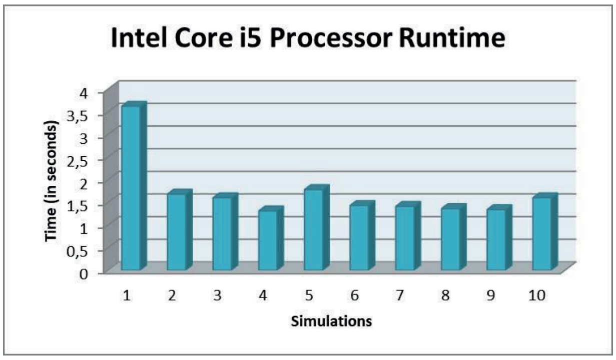

Figure 6: Time of execution of the samples submitted to the Hough Transform and Detection Color (HT-DC) algorithm on an Intel Core i5 processor

The average execution time of the images submitted to the HT-DC algorithm in computers with Intel Core i3 and Intel Core i5, corresponds to 1.4 and 1.7 seconds, respectively. The values of the hematimetric indexes VCM, HCM and CHCM obtained by the hematological equipment, common in laboratories of clinical analysis, were used as a parameter of comparison with the results obtained by the algorithm. Thus, it was possible to verify that the three functions implemented CVM, CHM, and CMCH presented 100% accuracy.

IV. Discussion

A high accuracy (90%) was obtained in the detection and counting of erythrocytes and leukocytes via the values obtained between the comparisons of the manual counts and the counts performed using the HT-DC algorithm. The same can be noticed through high accuracy (100%) presented by the function of the determination of hematimetric indexes. In this context, it is necessary to consider that the process of manually counting cells in blood smears, when performed repeatedly, becomes tiring and can lead to errors in counts. Such erroneous counts lead to an erroneous result, which can trigger an incorrect treatment for the patient.

In this way, the use of the HT-DC methodology can be seen as an important tool for both the health professional and the patient. In addition, larger laboratories with a higher demand for daily examinations that justify the acquisition of high-cost automated hematology equipment can use the HT-DC methodology as a confirmatory method for more altered exams released by the equipment conventional, thus conferring greater reliability to the medical reports issued.

One of the disadvantages of the automated methodology currently available is the need for specific equipment to perform the exams. These devices often present high acquisition cost, interface, maintenance, and require specific reagents for their operation, restricting the user to a specific company [4]. Therefore, in order to reduce equipment acquisition costs and consequently decrease the price of the final product, the HT-DC algorithm is able to provide a cheaper alternative than the current methodologies present in the market. In this way, with the reduction of the final price, it is possible to reach more widely the populations of developed and developing countries.

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Moreover, when using the HT-DC methodology, the healthcare professional has low cost, as this exempts the need for an hematocitometer and specific reagents for its operation: a mobile device, computer or notebook does the task.

From the patient's point of view, there is also a reduction in the final cost of service, since much of the burden of the exam is related to the type of methodology used. Another critical benefit to patients is the greater reliability and speed conferred to the hemograms, as these can be performed by a methodology that avoids counting and calculations failures. In addition, the rapidity of hematological examination is essential for the detection and indication of the most appropriate treatment of the individual [4]. Thus, runtime averages less than 4 seconds, prove that the HT-DC algorithm can be seen as an effective tool for laboratories and hospitals with a high demand for tests per day.

Considering the need for accessibility to health for all people, the process of counting blood cells by the HT-DC algorithm was performed on computers with different configurations. These devices were chosen because they are the most widely used today and provide more options for users who choose to perform blood cell counts through imaging methods. Considering the results of the simulations, it is noted that the accomplishment of hematological examinations can be carried out in the future in simple computers, complementing or even dispensing with the use of high-cost hematological equipment.

To verify the applicability of the HT-DC algorithm in different scenarios, the execution time of each of the digital blood smear images was analyzed. According to the work of Shoby et al. [19], the average execution time of each of the images was 4 seconds. Thus, the values presented in this research demonstrate an improvement of 35% on average in execution time on Intel Core i3 computers and 42.5% on Intel Core i5 machines, concerning works in the literature.

In addition, it is essential to consider that hematimetric indices are an important part of the erythrogram [2] [3]. Thus, the use of functions for its determination through a computer, provides more reliability to medical reports, because CVM, CHM, and CMCH are essential allies that indicate and/or detect the presence of anemia and leukemia.

In turn, the fact that the developed CVM, CHM, and CMCH functions receive a command that assists students and health professionals in the erythrocyte indices. By means of the "help" command, it is possible to display the name of each hematimetric index as well as its function in the blood count and its respective reference value. Thus, this methodology can be seen as a scientific-academic tool, which may also aid in the learning of medical students.

V. Conclusion

The confirmation of the diagnosis or even the accomplishment of laboratory tests through algorithms provides greater reliability of the results to both health professionals and patients because the algorithms reduce the chances of human failures. The good performance of the proposal on different hardware platforms concludes that the algorithm is feasible for the different realities of the laboratories.

The health area is a broad field directly linked to medical diagnoses through images, so the proposal of this study also predicts that the results suggestive of more serious pathologies can be stored in digital files for future consultations, dispensing with the creation of physical space, in this case for hospitals.

The function developed in this research to obtain the hematimetric indexes, as well as its immediate applicability can inform the students of the medical areas of the reference values and the applicability of each index.

In this way, the complete automation of the laboratory tests is a reality still distant for some laboratories of underdeveloped and developing countries. However, the creation of new methodologies, such as the one presented in this study, using image segmentation algorithms, results in a considerable reduction in equipment costs without loss in the quality and accuracy of hematological diagnoses.

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