MEDICINA E BIOMEDICINA

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(Organizadora)

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APRESENTAÇÃO

Em razão da coincidência do nome, muitos imaginam que Medicina e Biomedicina são áreas similares, ou ainda, concorrentes, mas a verdade é que médicos e biomédicos atuam em mercados de trabalho complementares, em conjunto, prezando pela qualidade de vida, prevenção, diagnóstico e tratamento de diversas patologias.

A Coletânea Nacional "Medicina e Biomedicina" é um *e-book* composto por 12 artigos científicos, que abordam relatos de caso, avaliações e pesquisas sobre doenças já conhecidas da sociedade, trata ainda da prevenção e detecção de patologias através da utilização de tecnologias já conhecidas e mostra ainda, o desenvolvimento de novas tecnologias para prevenção, diagnóstico, tratamento e monitoramento de outras enfermidades.

Enquanto os médicos têm seu foco voltado para a cura direta das doenças e restauração da saúde, os biomédicos voltam-se para o estudo, investigação e pesquisa das doenças. Os artigos elencados neste *e-book* contribuirão para esclarecer que ambas as profissões desempenham papel fundamental e conjunto para manutenção da saúde da população e caminham em paralelo para que a para que a ciência continue evoluindo para estas áreas de conhecimento.

Desejo a todos uma excelente leitura!

Lais Daiene Cosmoski

SUMÁRIO

CAPÍTULO 6

COUNTING OF ERYTHROCYTES AND LEUCOCYTES THROUGH THE DIGITAL IMAGE SEGMENTATION ALGORITHM WT-MO: A QUICK AND LOW-COST METHODOLOGY

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ABSTRACT : Considering that a large part of the world population today is concentrated in underdeveloped and developing countries, and that access to health is a right guaranteed by the Constitution, creating new, more accessible diagnostic methods is a challenge for the medical areas. The necessity for practicality, reliability, and agility has stimulated the creation of new tools for the health area. The blood cell counting is an important exam that helps to diagnose various diseases besides to be an important field of study in biomedical engineering. Currently, the red blood cells count and white blood cells have been performed by the automated and manual method. However, still does not dispense the use of the manual method, through the preparation of blood smears, in specific cases or only for confirmation of diagnostics. In the last years, Matlab® software has been used to develop algorithms to facilitate cell counting. Thus, the present work implements an algorithm based on Watershed transform, where its results show a better performance of 34% in execution time in comparison with other works developed, as well as a computational performance of 1.98 seconds, as also showing high reliability in your digital count.

KEYWORDS : Hematology, Watershed Transform, Digital Imaging Processing, Biomedical Engineering.

1 I INTRODUCTION

Human blood consists of plasma and cells (erythrocytes, leukocytes and platelets). The erythrocytes are biconcave disc-shaped anucleated cells responsible for transporting oxygen through the body. These cells are also called red blood cells or red blood cells, and measure under normal conditions on average 7µm in diameter (MONTEIRO *et al*, 2015; TURGEON, 2004). The red blood cell count in non-pathological conditions should be expressed from 4.0 to 6.0 x $10^6/mm^3$ of blood, suffering slight variations according to patient's age, sex and lifestyle (MOHAMMED *et al*, 2013; SAHASTRABUDDHE; AJIJ, 2016).

Reduction in the amount of erythrocytes is called erythropoiesis. When this occurs there is suspicion of blood loss or anemia. In turn, the increase in the number of red blood cells is called erythrocytosis, being directly related to emergence of polycythemias, which can progress to severe complications such as stroke or infarction, that may lead to death (BERNADETTE; RODAK; FRISTMA, 2015).

Leukocytes are also called white blood cells. These cells are directly linked to innate and acquired immunity, having as a function the defense of the organism. In healthy individuals, leukocytes have a blood count of 3.500 to 10.500 leukocytes/ mm^3. Leukocytes expressed in amounts above the reference value are indicative of leukocytosis. When this occurs there is suspicion of acute and/or chronic inflammations or infections by external etiological agents, such as allergic agents, helminths, viruses, bacteria, among others (BERNADETTE; RODAK; FRISTMA, 2015; TURGEON, 2004; MAZALAN; MAHMOOD; RAZAK, 2013).

In this context, blood cell counts can be made by automated or manual methods. Automated methods are characterized by higher speed, however, they are more costly. This is because, the hematological devices are based on the principles of impedance pulses, flow cytometry, absorbance, among others (FAILACE *et al.*, 2009; SOBHY; SALEM; DOSOKI, 2016).

However, the existing automatic methods do not negate the use of manual methods, as there are times when these devices are unable to analyze blood samples or require confirmation of the reports issued. This occurs in the following cases: children under 5 years and patients 75 years or older; cancer patients; patients in severe condition; patients with leukocytosis or leukopenia, cases of color variations, erythrocyte size and shape, among others ((FAILACE *et al.*, 2009).

In contrast, manual methods are characterized by lower prices, but require more time for the tests. This occurs, because this methodology requires the making of blood smear (FAILACE *et al.*, 2009; TURGEON, 2004). Only the final portion of the slide is used to perform counting, because the anterior portions are composed of clustered and/ or overlapping cells, preventing a reliable count. In addition to counts and observations of red cell morphologies, health professionals perform calculations to complete the diagnoses, which generally take into account total erythrocyte and leukocyte counts (BERNADETTE; RODAK; FRISTMA, 2015; TURGEON, 2004).

In recent years, many methodologies have been developed to facilitate and solve problems in the health field. The studies (ARIVU; SATHIYA, 2012; MAZALAN; GUITAO *et al.*, 2009; HEIDI *et al.*, 2011; HEMAN; SEXENA; VADAK, 2013; KAUR; KAUR, 2014; MAHMOOD; RAZAK, 2013; MOGRA; SRIVASTAVA, 2014) has used digital image processing techniques through Matlab software, aiming to perform counts of cells present in blood smears. In these studies, extraction and counting were based on the morphology of blood cells through the distance found between each pixel; or through the Hough Transform by performing the red cell count by detecting geometric features, finding the circular center of the image; or segmentation of erythrocytes in order to determine the cell boundary, among others. However, some techniques result in poor performance and low accuracy when applied in smears with large amounts of cells.

Given the complexity of medical imaging, it is necessary to create a systematic segmentation that meets the criteria of accuracy and reliability (CUEVAS *et al*, 2013). In this way, the implementation of digital image processing of blood cells is responsible for assisting in clinical decisions, through obtaining results in a faster, easier and more agile way, besides reducing costs with the acquisition of equipment.

The creation of a new methodology for counting blood cells with reduced costs has impacts directly related to health professionals and patients. Cost-effective equipment stimulates the entrepreneurship of newly trained professionals, as well as making blood cell counts less tedious and less susceptible to erroneous counts. From the point of view of patient health, methodologies such as these make the medical reports even more reliable and bring the benefit of reducing the amount paid for each exam, since the reduction in the cost of the methodology directly impacts the reduction of the value of the final product/service. Such a reduction may make the blood count more accessible to less favored populations, and may make it possible for these people to detect anemia and/or leukemia early in their early stages.

In this way, the Watershed Transform proposes a morphological approach to the image segmentation problem, and can be applied in several areas of knowledge. This technique interprets the images where each pixel corresponds to a position and the gray levels relative to each of these pixels determine their altitudes, being a powerful tool found to solve the problems addressed in this research (CUEVAS, 2013; GONZÁLEZ; WOODS, 2002; HEMANT; SEXENA; VADAK, 2013; MOHAMMED *et al,* 2013; SOBHY; SALEM; DOSOKI, 2016, VINCENT; SOILLE, 1991).

The algorithm presented in the present study has the objective of reaching all these points, solving them in less processing time and computational cost associated with high accuracy in the detection of erythrocytes and leucocytes, as well as a better execution performance in comparison to other works as (ARIVU; SATHIYA, 2012; GUITAO *et al*., 2009; HEIDI *et al*., 2011; MAHMOOD; MANSOR, 2012; MAZALAN; MAHMOOD; RAZAK, 2013; MOGRA; SRIVASTAVA, 2014; SAHASTRABUDDHE; AJIJ, 2016; SOLTANZADE *et al,* 2010).

2 I METHODOLOGY

The algorithm developed has the function to perform the counts of red cells and leukocytes through a digital image of a peripheral blood smear. The processes of counting and differentiation of blood cells are performed based on the recognition of cell nuclei, size differentiation in pixels and color intensity present in each type of cell analyzed. Following the segmentation and image processing logic, the algorithm is executed, with accuracy objectives and computational costs.

The experiments were conducted by blood smears, stained and analyzed under an optical microscope under the objective lenses of 40 and 100, resulting in an image increase of 400 and 1000 times, respectively. Later, through a camera coupled to an optical microscope, digital images were acquired from the fields of the slide containing red blood cells without agglomerations and presence of leukocytes. These images were acquired in the digital formats “png”, “jpeg” and “jpg”, being transferred to a computer, where previously there was been installed the software Matlab, version 8.3 of 64 bits (2014a). This tool was chosen because it is already consolidated in the scientific environment, having its libraries and structure already tested and validated. The logic of the development and performance of the algorithm is represented in the diagram of Figure 01.

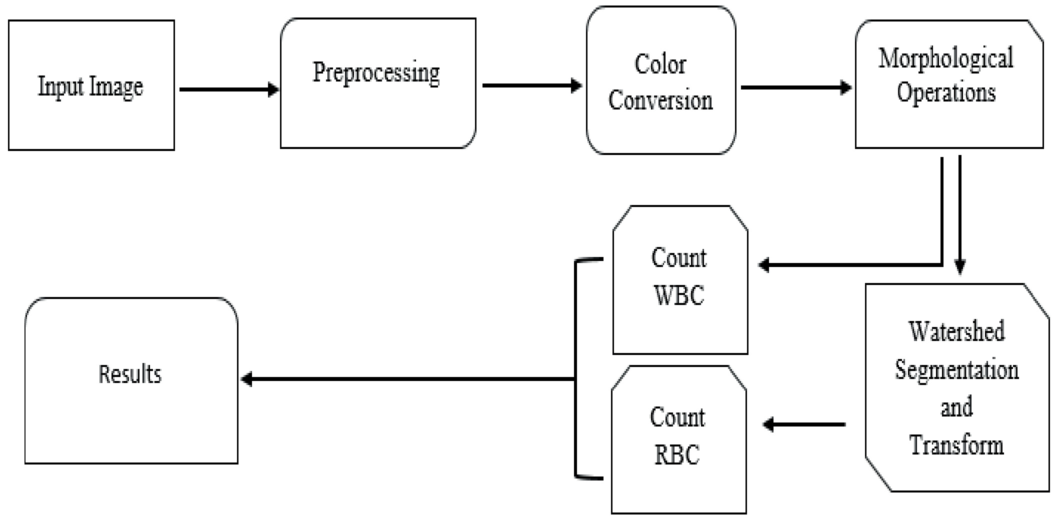

Figure 01 – Diagram representing the WT-MO algorithm in erythrocyte and leukocyte counts

After the input of the image in the Matlab software simulation environment, there was a preprocessing step, which involves some steps such as filtering and image processing, that has the purpose of solving lighting problems usually arising from the moment of acquisition of the images through optical microscopy. In terms of quality for the next steps of the algorithm are the conversion of RGB images (“Red, Green and Blue”) to gray scales and binary scales. After this phase, the Watershed Transform and morphological operations were used. The union of these two techniques of segmentation of digital images for the purpose of detection and counting of blood cells, originated the algorithm WT-MO, being WT derived from the abbreviation “Watershed Transform” and the MO from “Morphological Operations”.

The stage of morphological operations is used to segment the leukocytes, present in the digital images. In this process, each of the white blood cells found is removed from the image in order to avoid being erroneously counted as erythrocytes. This process is based on the concepts of size, shape, structure and connectivity of objects in the image, involving the steps of erosion, dilation, opening, closing and reconstruction of the digital image. Dilation consists of adding pixels to the edges of objects in an image, in order to repair breaks in the image. Erosion is responsible for removing the pixels present at the edges of the image, being used to divide objects. The opening is applied to smooth out contours. The closing is used to merge intervals and fill spaces. The reconstruction is responsible for extracting relevant information from the image (VINCENT; SOILLE, 1991).

Subsequently, the digital image of the blood smear passes through the erythrocyte count, where the cells are segmented and counted through the Watershed Transform. In this step, each counted red cell receives a number according to its count by the algorithm. The Watershed Transform also acts in the processing, calculation and recognition of distances between nuclei, avoiding possible erroneous counts of overlapping cells. Finally, the scores results are released separately. Figure 2 shows the performance of the WT-MO algorithm in the red cell and leukocyte count.

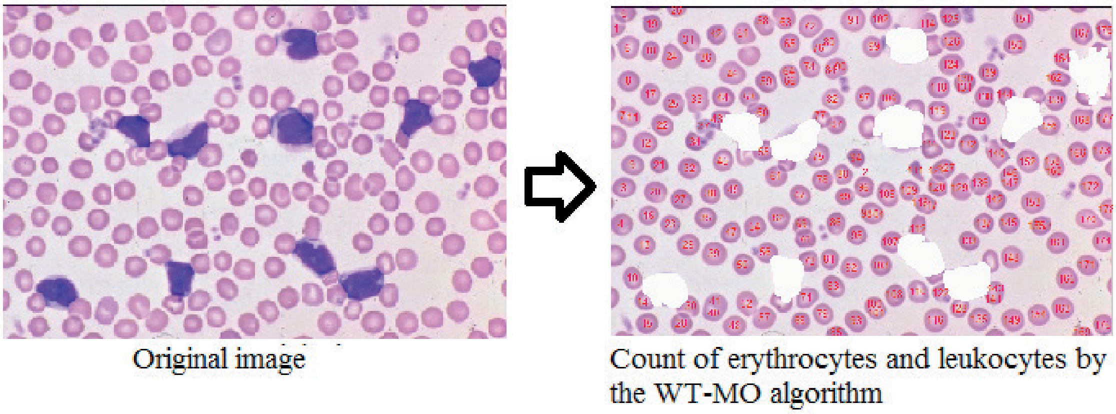

Figure 02 – Running the algorithm in a sample

3 I RESULTS AND DISCUSSION

In this section, the results of the WT-MO algorithm will be presented through evaluations of accuracy, processing and execution time. For this study, 18 digital images containing red blood cells and leukocytes were used. These samples were submitted to manual counts by a biomedical professional, and were subsequently counted by the WT-MO algorithm. The results obtained presented better accuracy when compared to the (ARIVU; SATHIYA, 2012; GUITAO *et al.*, 2009; HEIDI *et al.*, 2011; MAHMOOD; MANSOR, 2012; MAZALAN; MAHMOOD; RAZAK, 2013; MOGRA; SRIVASTAVA, 2014; SAHASTRABUDDHE; AJIJ, 2016; SOLTANZADE *et al,* 2010), as shown in Figures 03 and 04.

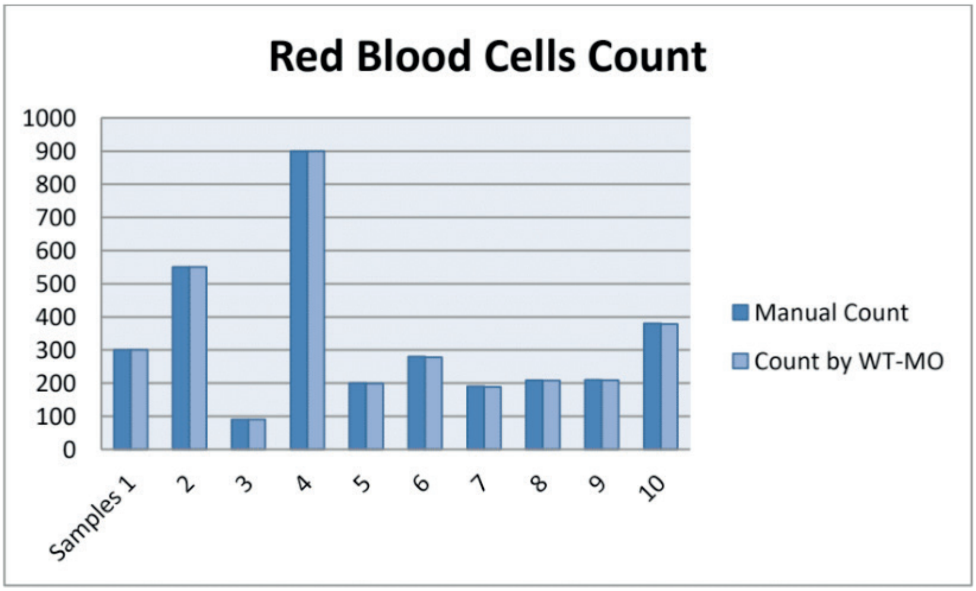

Figure 03 – Erythrocyte Count Through Manual Methodology x WT-MO Algorithm

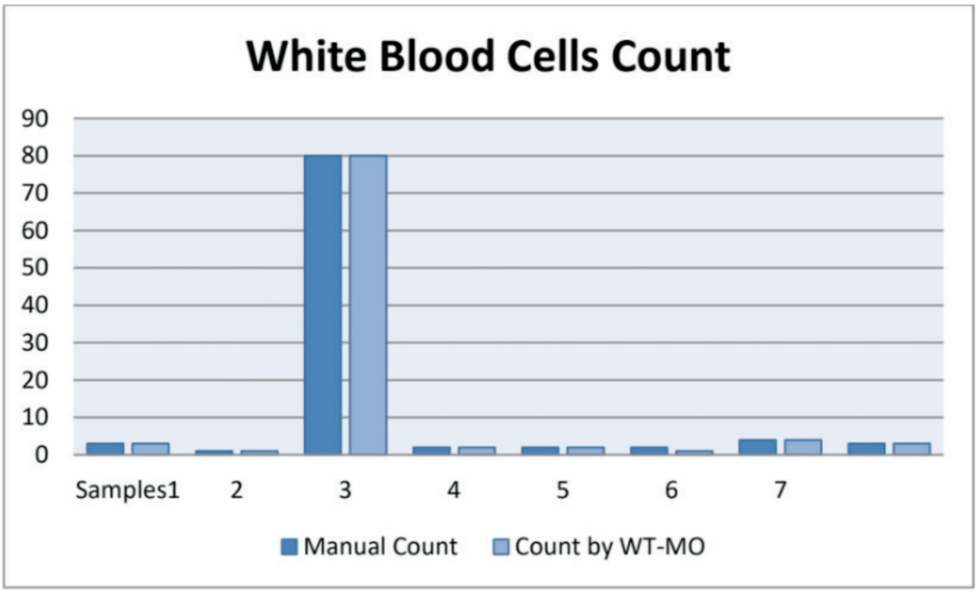

Figure 04 – Leukocyte Count Through Manual Methodology x WT-MO Algorithm

To evaluate processing time and execution time, simulations were performed on two different hardware platforms, consisting of an Intel Core i5 processor with 8GB RAM and another with an Intel Core i3 processor with 4GB RAM. These configurations were chosen because the purpose of this study is to create an algorithm that is capable of being executed in several hardware platforms, dispensing with the need of acquisition of a specific equipment for the counting of blood cells. In this context, the Intel Core i3 and i5 processors were chosen because they are the most commonly available on the market, currently.

The “tic, toc” and “sim” commands were used via the command line at the Matlab software prompt. This command has the function of measuring the execution time, that is, it quantifies the time required to execute the algorithm (in seconds). Also used the "cputime" command, which is responsible for quantifying the processing time of the algorithm. This command acts by returning the total CPU time (in seconds) used by the algorithm, being considered from the moment the process as a whole was started. Together, they show the performance and efficiency of the WT-MO algorithm. Thus, the first execution of the algorithm was analyzed in the described commands, since it is in its first execution that the variables are allocated and the memory reserved for their execution, referring to Figures 05 and 06.

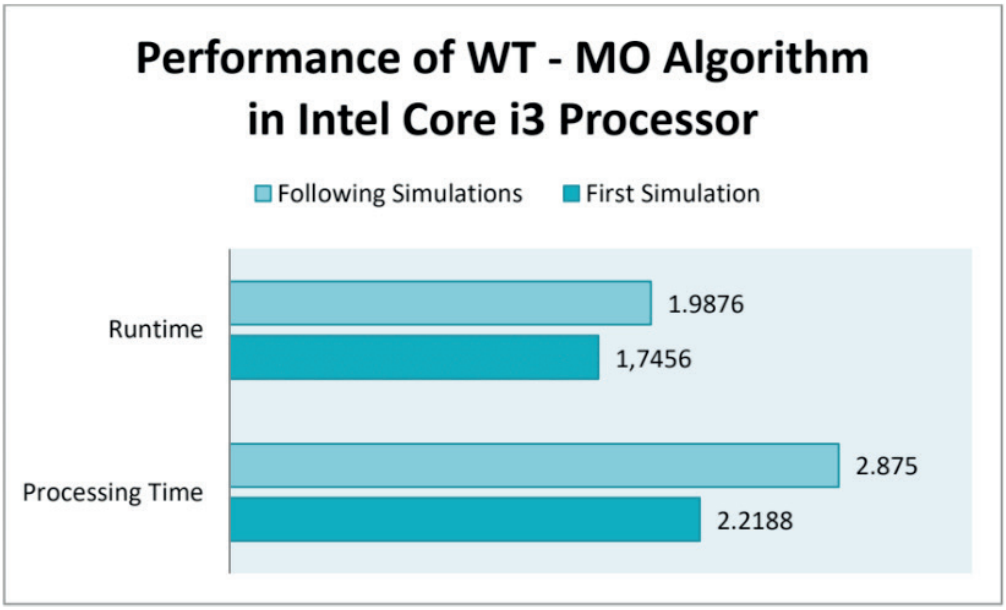

Figure 05 – Performance by the WT-MO algorithm by the Intel Core i3 processor

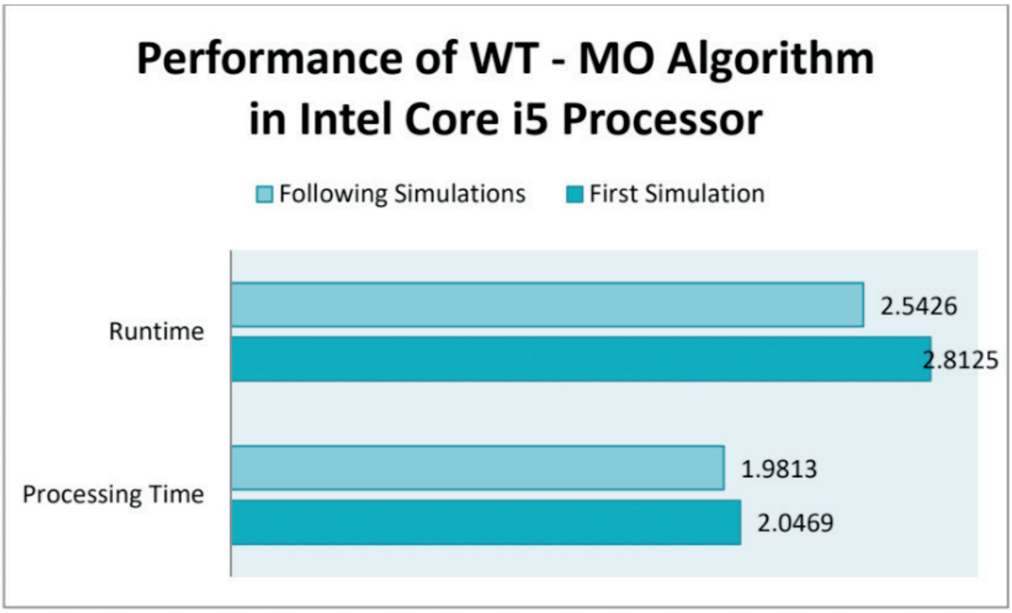

Figure 06 – Performance by the WT-MO algorithm by the Intel Core i5 processor

According to the paper (MAHMOOD; MANSOR, 2012), regarding blood cell detection through algorithms based on image processing, a runtime of 3 seconds per sample was obtained. However, the WT-MO algorithm for detecting and counting erythrocytes and leukocytes, as seen in Figures 05 and 06, obtained a better performance of 15.3% in its first execution and 34% in the following ones, which correspond in time to 2,54 seconds and 1,98 seconds, respectively, the average time of the first run of all samples. These results refer to executions performed on an Intel Core i5 computer, according to the data presented in Figure 06.

Executions performed on an Intel Core i3 computer also showed more satisfactory results than those found in the study (MAHMOOD; MANSOR, 2012), wherein its first execution, there was a 34% improvement in performance, and 42% in the following, corresponding to 1,98 seconds and 1,74 seconds, respectively, also consisting of the average time of all samples. These results are presented in Figure 05.

Considering that humans are distinct from each other, consequently, the results of their laboratory examinations are as well. This variation is due to the type of pathology of the patient, which can cause differences in size, color and quantity of red blood cells and leukocytes. In this way, 4 images of the total images were selected in order to analyze the performance of the WT-MO algorithm in samples with different characteristics, such as size, the quantity of red cells and leukocytes. In this case, the first run analysis was not considered, only the performance of the algorithm was analyzed. The results are shown in Figures 07 and 08.

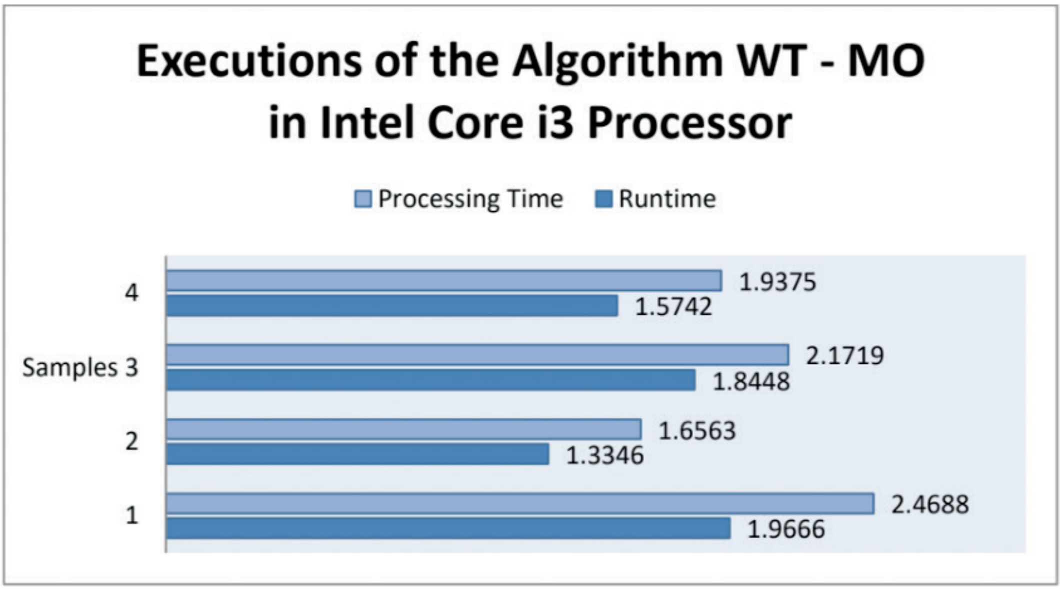

Figure 07 – Executions of the WT-MO Algorithm by the Intel Core i3 Processor (in seconds)

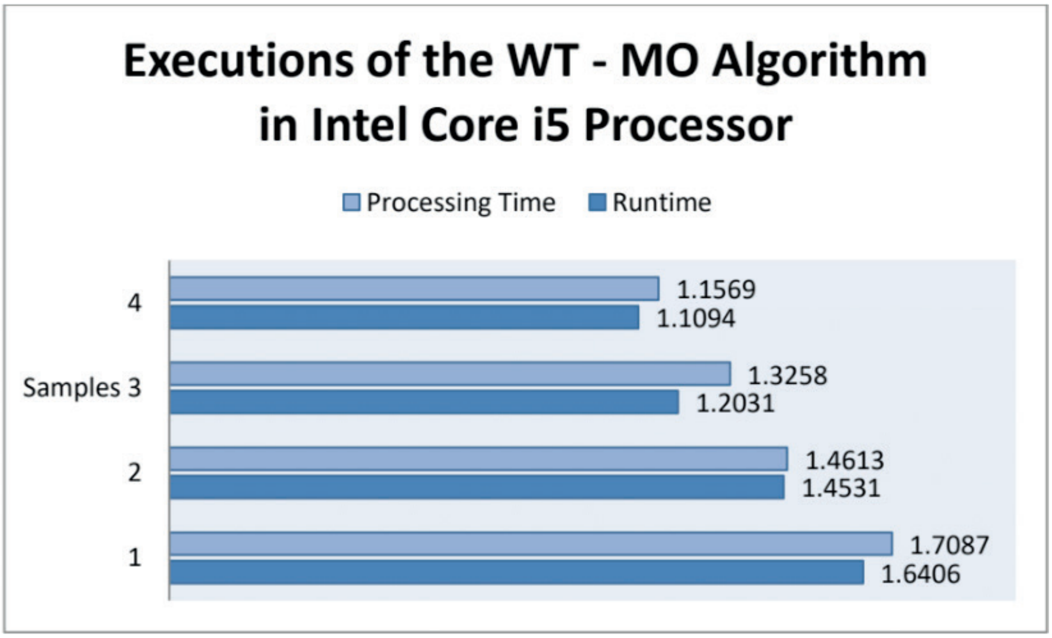

Figure 08 – Executions of the WT-MO Algorithm by the Intel Core i5 Processor (in seconds)

Analyzing the results presented in Figures 07 and 08, it is noted that the processing time in Intel Core i3 computers was higher, such factor is due to the Intel Core i3 processor having 2 cores less when compared to the Intel Core i5 processor. In addition, computers with an Intel Core i5 processor has twice the RAM. However, even with these differences, the results are still satisfactory in relation to the performance of the proposal of this research. The executions presented in Figures 07 and 08 were compared with the study (HEMANT; SEXENA; VADAK, 2013), where Table 1 shows these results as the best performance.

Samples	***Study***	***WT-MO***	
	Hemant	***i3***	***i5***
1	12,72”	84,54%	86,57%
2	2,61”	48,87%	44,01%
3	2,72”	32,18%	51,26%
4	4,76”	66,93%	75,70%

Table 1 – Performance Improvement WT-MO

It is important to note that in hospital environments, time is an extremely important variable because the faster the test result is obtained, the faster the patient can be referred for treatment or complementary tests. This fact justifies the analysis of processing time and execution time used to analyze the WT-MO algorithm for counting erythrocytes and leukocytes. In cases of patients with leukocytosis, even if the hemogram has been performed on hematological equipment, it is recommended that these reports be confirmed by performing a blood smear and manually counting the cells. The WT-MO image segmentation algorithm can be used as a complementary diagnostic method, with the release of results faster than those released by manual methodologies.

4 I CONCLUSIONS

In all the analyzed scenarios and in all samples, the WT-MO algorithm obtained better results of execution time and processing time when compared to previous work done by other authors, being also able to solve the problems encountered during the counts of red blood cells and leukocytes. In addition, the WT-MO algorithm has performed satisfactorily, both in efficiency and reliability, which are paramount during the completion of a laboratory diagnosis.

The good performance of the proposal in computers with different hardware configurations 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 a physical space, in this case for hospitals. The development of this efficient algorithm for counting red cells and leukocytes through image processing and segmentation techniques contributes to medical studies and can be considered the first step to make the hemogram a more accessible examination for the less favored populations, and is more accessible to professionals who opt for entrepreneurship in the areas of clinical analysis, since the WT-MO algorithm requires the acquisition of high-cost hematological devices, being dependent only on the acquisition of a computer.

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