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“MATLAB algorithm and VHDL modules for morphologic detection, recognition and classification of cells in clinical analysis for implementation on FPGAs: its design and development.”

By

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Master of Science

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ABSTRACT

Laboratory tests and clinical analysis is a must to confirm or reject some medical diagnosis. Common analytical fields of knowledge are: Hematology, Clinical biochemistry, Urology, Microbiology among others. This work is for the clinical field of Hematology where detection of immature leukocytes on peripheral blood sample is part of a clinical test of relevance for detection of certain types of cancer.

Hematologists distinguish metamyelocytes by their characteristic kidney shape and band Neutrophils by C or S shape of their nucleus.

This work focuses on the development of an algorithm in MATLAB, for detection, recognition and classification of abnormal White Blood Cells (WBC) in peripheral blood samples and implementation of some VHDL modules in a FPGA; with the purpose to ease future applications in devices for clinical analysis.

Development and implementation could contribute to improve some devices, like blood analyzers based on microscopy augmented images.

RESUMEN

Las pruebas de laboratorio y análisis clínicos son un requisito indispensable para confirmar o descartar algunos diagnósticos médicos. Los campos analíticos más comunes en esta área son: Hematología, Química Clínica, Urología, Microbiología, entre otros. Este trabajo está ubicado en el campo clínico de Hematología, donde la detección de leucocitos inmaduros en muestras de sangre periférica es parte de una prueba clínica de relevancia para la detección de ciertos tipos de cáncer.

Hematólogos distinguen metamielocitos debido a la característica forma arriñonada y los neutrófilos de banda debido a la forma C o S de sus núcleos.

Este trabajo se enfoca en desarrollar un algoritmo en MATLAB, para la detección, reconocimiento y clasificación de glóbulos blancos anormales en muestras de sangre periférica y algunos módulos en VHDL para implementarlos en un FPGA, con la finalidad de facilitar futuras aplicaciones en dispositivos para análisis clínicos.

El desarrollo e implementación podría contribuir para mejorar algunos dispositivos, como lo son los analizadores de sangre que trabajan sobre imágenes de microscopio aumentadas.

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Many thanks to all my friends and people who have been around these two years, who have been helping me at different occasions and in one way or another, have influenced this thesis.

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I dedicated this thesis to the memory of my grandmother

Aurelia Sánchez Romero

For being an example of strength and love

CONTENTS

ABSTRACT	ii
RESUMEN	iii
ACKNOWLEDGMENTS	iv
Chapter 1 INTRODUCTION	1
1.1 Motivation	1
1.2 Previous Work	2
1.3 Problem description	4
1.4 Objectives	5
1.4.1 <i>General</i>	5
1.4.2 <i>Particular</i>	5
1.5 Solution Approach	6
1.6 Summary	8
Chapter 2 CONCEPTS OF RELEVANCE FOR THE ALGORITHM DEVELOPMENT	9
2.1 Introduction	9
2.2 Manual Peripheral Blood Smear Examination by Hematologists.	9
2.2.1 <i>Preparation of peripheral blood smear</i>	10
2.2.2 <i>Staining of Peripheral Blood Smears</i>	11
2.2.3 <i>Peripheral Smear Examination</i>	12
2.3 Leukocytes Count	13
2.3.1 <i>Automated leukocyte count</i>	14
2.3 Cells of Interest	15
2.4 Summary	16
Chapter 3 ALGORITHM DEVELOPMENT USING MATLAB	17
3.1 Introduction	17
3.2 Nucleus Segmentation	18
3.3 Nucleus Pre-processing	19
3.4 Feature extraction	22
3.5 Recognition and Classification	23

3.5.1 <i>Band Neutrophil Recognition</i>	24
3.5.2 <i>Metamyelocyte Recognition</i>	27
3.6 Summary.....	30
Chapter 4 VHDL IMPLEMENTATION	31
4.1 Introduction	31
4.2 VHDL Algorithm Implementation	31
4.3 Design Entities Description.....	33
4.3.1 <i>Count and Shift Entity</i>	33
4.3.2 <i>Maximum Ones Entity</i>	35
4.3.3 <i>Minimum Ones Entity</i>	36
4.3.4 <i>RAM Memory</i>	37
4.4 Summary.....	38
Chapter 5 TESTS AND RESULTS	40
5.1 Introduction	40
5.2 MATLAB Algorithm Results	40
5.3 VHDL Implementation Results.....	46
5.3.1 <i>Synthesis Results</i>	46
5.3.2 <i>FPGA Programming Results</i>	51
5.4 Summary.....	54
Chapter 6 CONCLUSIONS AND FUTURE WORK	55
6.1 Conclusions.....	55
6.2 Future Work	56
FIGURES LIST	57
TABLES LIST.....	60
Appendix A.....	61
A.1 Results displayed by 3 Automated Blood Counting Instruments.....	61
GLOSSARY.....	64
REFERENCES	66

Chapter 1 INTRODUCTION

1.1 Motivation

Clinical analysis is of most relevance as support for medical diagnosis, which consists of a requested test by some medical doctor to a laboratory in order to confirm or reject a diagnosis. Some analytical fields of these tests are for example: Hematology, Clinical biochemistry, Urine chemistry, microbiology and genetics among others.

In this work hematology is our area of interest, which can be defined as a branch of the medical science that studies blood structural elements and their predecessors (immature forms), as well as the structural and biochemical disorders of these elements that could lead to a disease; for example, different types of cancer (leukemia, renal and hepatic among others). The hematologic diseases affect the blood production and its components like red blood cells (erythrocytes), white blood cells (leukocytes), hemoglobin, plasmatic proteins, the coagulation mechanism, etc [1].

Hematology includes cell package study, blood profile or state, which are: erythrocytes count, leukocytes count, hemoglobin determination, globular sedimentation speed, leukocytes differential count, among others.

Nowadays automatic analyzers have increased the efficiency and speed of the laboratory analyses, even with these advances there is need to include a quantitative and qualitative analysis of abnormal cells, to improve the range of these devices [2].

1.2 Previous Work

For a summary of previous works, see Table 1-1. This was done as a result of our previous work review, this considers 6 elements shown as columns, which are:

- Reference
- Techniques,
- Sample Type
- Type of cells,
- Atlas' Classification [3],
- Results and future work,

Technique's column, mentions the different techniques used by the reference, for example, fuzzy logic, neuronal networks, etc.

Sample type establishes whether peripheral blood or bone marrow was used as blood sample.

The third and the fourth columns are for the type of cells classified in the references and the classification of them by the atlas. Then the results' column comments some results of the classification algorithms proposed by the references and finally the last column talks about future work propose by the authors.

For some of the papers there are empty columns because the corresponding information is not included on the work.

Reference	Techniques	Type of sample	Type of cells	Classification by Atlas	Results	Future Work
[4]	Method to segment nucleus and cytoplasm. Using morphological operators	Peripheral blood	White blood cells (WBC)	Band Neutrophil, Lymphocyte, Monocyte, Polymorphonu clear neutrophil.		
[5]	Neural Networks.	Peripheral Blood	Erythrocytes Leukocytes and platelets		The final recognition rate of testing set is: 88.9%	To improve the performance, other kinds of features And additional decision rules may be introduced.
[6]	Fuzzy techniques	Bone Marrow	White Blood Cells in bone marrow.			
[7]	Quantitative measurements of size, Curvature, and color of the nucleus and cytoplasm.	Peripheral Blood	White blood cells Basophil, Eosinophil, lymphocyte, Monocyte, Neutrophil.	Lymphocyte, Monocyte, Polymorphonu clear neutrophil, Basophil, Eosinophil.	Rate close to 91%.	To be solved in the case of overlapping and Touching cells.
[8]	Morphological operators	Peripheral Blood	Red blood cells			
[9]	Neural network based classifiers and support vector machine	Bone Marrow	White Blood cells Erythroblast, Lymphoblast, Metamyelocyte , Monoblast, Myeloblast, Myelocyte, Plasma cell, Proerthroblast, Promyelocyte, Band, and Megakaryocyte		Classification of cells with SVM. Its performance is found to be 87.98 %.	We are working on evaluating classifier Combinations such as committees of networks and stacked generalization to improve the robustness of the classification step.
[10]	Method based on deformable shape models for medical image segmentation	Peripheral blood	Blood cells			Classification by cell inclusions, quantitative Analysis of cell types, and blood smear image indexing and Retrieval.
[11]	Scale-space filtering and Watershed clustering	Peripheral Blood	White blood cell (WBC)	Band Neutrophil, Lymphocyte, Monocyte.	Average "entirety" of 95.3% and an average "accuracy" of 98.9%.	
[12]	Color Image processing	Peripheral	White Blood Cells (WBC)	Monocyte	A segmentation accuracy Of 80%. The best	

					classification accuracy of 97% is obtained using Neural Networks, followed by 94% using SVM.	
[13]	Morphological analysis, Neural Classifiers		White blood cells Basophil, Eosinophil, Lymphocyte, Monocyte and Neutrophil.	Lymphocyte, Monocyte, Polymorphonu clear neutrophil, Basophil, Eosinophil.		Identification of tumor deformations in the cell morphology For a fully-automated diagnostic system.
[14]	Texture Processing, Statistical Pattern Recognition	Peripheral Blood	Leukocytes, Neut, lymph, eosi, mono, baso.	Band Neutrophil, Lymphocyte, Monocyte, Basophil, Eosinophil.		
[9]	An eight-dimensional multivariate Gaussian Classifier	Peripheral Blood	Small Lymphocytes, Medium Lymphocytes, Large Lymphocytes, Monocytes, Band Neutrophils, Segmented Neutrophil, Basophils, Eosinophils.	Polymorphonu clear neutrophil, Band Neutrophil, Lymphocyte, Monocyte, Basophil, Eosinophil.	93 % of the testing set was classified correctly by the computer	Involving larger clinical studies, with blood samples from different population compositions would be required to test the reliability of these methods.

Table 1-1 Summary of Previous works.

1.3 Problem description

Several works about blood cell detection, recognition and classification are only focus on the diagnosis of some specific disease like cancer, and malaria by means of the cell morphology and their quantity. These consist of developing algorithms for the cell classification of certain cell type, some techniques used in these algorithms are: Fuzzy Logic, Neuronal Networks, morphologic operations and Wavelets among others.

From the reviewed references, there is no mention to the existence of algorithms for the recognition of immature forms on peripheral blood; although exist some works about immature forms on bone marrow such as [15], [16]. Automated blood analyzers based on flow cytometer differentiate the WBCs into five subpopulations: *Neutrophils*, *Lymphocytes*, *Monocytes*, *Eosinophils*, *Basophils* [17] Automated blood analyzers do flag abnormal conditions on WBC count that require manual laboratory review by an hematologist. This work is focused towards those applications that can make use of images of peripheral blood samples, and that are flagged for manual review by the automated blood analyzer. This opens the opportunity to contribute on design and implementation for solutions based on FPGA devices. Appendix “A” shows sample reports from typical automated blood analyzers

1.4 Objectives

1.4.1 General

To design an algorithm, for detection, recognition and classification of abnormal White Blood Cells (WBC) in peripheral blood samples with some VHDL modules to implement them in a FPGA, in order to facilitate future applications in devices for clinical analysis.

1.4.2 Particular

- Design al algorithm to extract characteristics from every image, for their later recognition and classification, in Matlab.
- Develop VHDL modules for the previous algorithm to implement them in a FPGA prototype.

- Ease the VHDL module development that will synthesize into an FPGA device.

1.5 Solution Approach

This thesis develops an algorithm in Matlab and their partial VHDL I/O equivalents, for *detection*, *recognition* and *classification* of 2 types of immature white blood cells, by means of image processing, analyzing the morphology of their nucleus. The basis for knowledge acquisition is a hematology atlas [3], given that atlases are a common reference source for clinical lab hematologists to classify blood cells; additionally some other texts [18] [19]. These sources use cell's morphology of their cellular components to classify them.

Figure 1-1 shows the MATLAB algorithm development process; starting from a peripheral blood smear image from a microscope, the nucleus is segmented and process morphological features in order to classify the cells; while the classification is not the expected one, the process will be repeated until a equivalent classification from the specified on a hematologic atlas is obtained

During the design and development of the MATLAB algorithm a special effort was placed to ease the VHDL module development that will synthesize into an FPGA device.

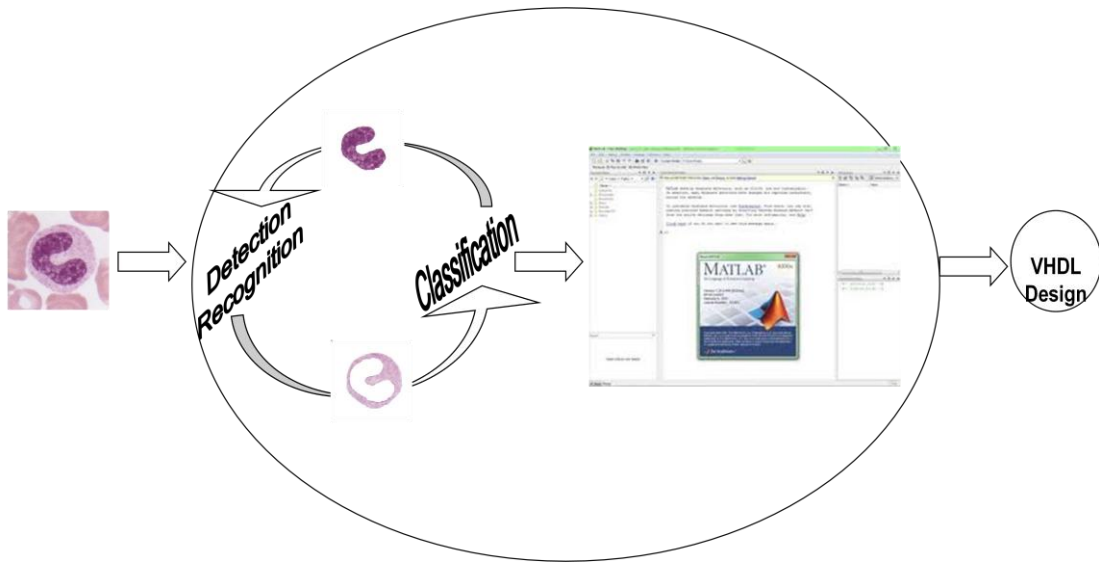


Figure 1-1 MATLAB Algorithm development process.

After completing the MATLAB version, we will design and develop enough modules in VHDL to show applications that classify two different abnormal white blood cells from peripheral blood samples. Once the modules are designed, these will be synthesized and tested on a FPGA. The set of modules opens the possibility to develop *detection*, *recognition* and *classification* of other abnormal blood cells by defining different module interconnections and possibly other new modules.

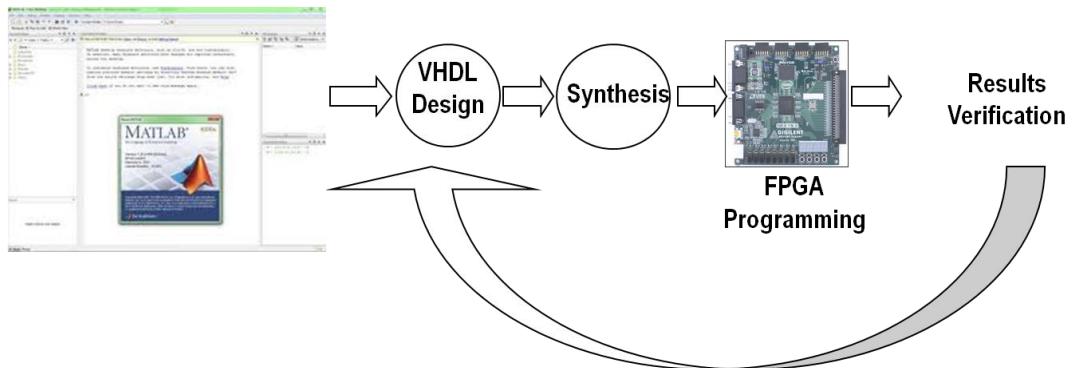


Figure 1-2 VHDL Algorithm development process.

Classified images by specialists will be processed, for the system classification. Verification of functional equivalence will be performed by using classified images by specialists and comparing results with the obtained from the FPGA implementation. In the implementation stage we expect the same results as in Matlab.

1.6 Summary

This chapter discussed the lack of published research on recognition and detection of immature cells into **peripheral blood** samples.

The motivation, problem and objectives for this work were described.

Next chapter will be focused on the concepts for the correct development of this work, as the importance of cells' features and the leukocytes count.

Chapter 2 CONCEPTS OF RELEVANCE FOR THE ALGORITHM DEVELOPMENT

2.1 Introduction

This thesis work uses some hematology concepts of importance into, as well as the cells' features used by clinical hematologists to identify some immature cells. These concepts will be described below.

2.2 Manual Peripheral Blood Smear Examination by Hematologists.

Examination of the peripheral blood smear should be considered, along with review of the results of peripheral blood counts and red blood cell indices, an essential component of the initial evaluation of all patients with hematologic disorders. The examination of blood films stained with Wright's stain frequently provides important clues in the diagnosis of anemias and various disorders of leukocytes and platelets.

Examination is commonly used to supplement the information provided by automated hematology analyzers ("blood cell counters"). Hematology analyzers provide accurate quantitative information about blood cells and can even identify specimens with abnormal cells. However, the precise classification of abnormal cells requires a hematologist, a well-made peripheral blood smear, and a light microscope with good optical characteristics.

2.2.1 Preparation of peripheral blood smear

The wedge smear is the most convenient and commonly used technique for making peripheral blood smears. The steps for the preparation of peripheral blood smear are listed below:

1. Placing a small drop of venous blood on a glass microscope slide, using a glass capillary pipette. A wooden applicator stick can also be used for this purpose.
2. A spreader slide has been positioned at an angle (30° to 45°) and slowly drawn toward the drop of blood.
3. The spreader slide has been brought in contact with the drop of blood and is being drawn away. Note layer of blood at the edge of the spreader slide. The spreader slide is further pulled out, leaving a thin layer of blood behind.
4. End result. A glass slide with a well-formed blood film. After drying for about 10 minutes, the slide can be stained manually or placed on an automated slide stainer.

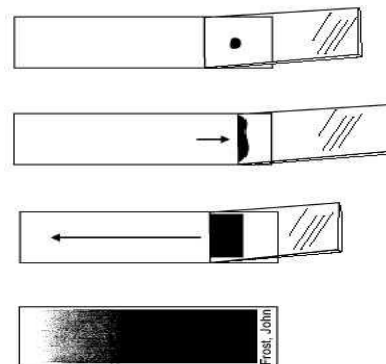


Figure 2-1 Peripheral Smear Preparation

A well-made peripheral blood smear has the following characteristics [3]:

- About two thirds to three fourths of the length of the slide is covered by the smear.
- It is slightly rounded at featheredge (thin portion), not bullet shaped.
- Lateral edges of the smear should be visible. The use of slides with chamfered (beveled) corners may facilitate this appearance.
- It is smooth without irregularities, holes, or streaks.
- When the slide is held up to light, the featheredge of the smear should have a “rainbow” appearance.
- The whole drop is picked up and spread.

2.2.2 Staining of Peripheral Blood Smears

The purpose of staining blood smears is to identify cells and recognize morphology easily through the microscope. Wright stain is the most commonly used. These contain both eosin and methylene blue. Free methylene is basic and stains acidic cellular components, blue. Free eosin is acidic and stains basic components, red.

A well-stained slide is necessary for accurate interpretation of cellular morphology [3].

2.2.3 Peripheral Smear Examination

Examination of the blood smear is a multistep process. These steps are described below [3] [18]:

- *10x Examination*, this step is necessary to assess the overall quality of the smear, including abnormal distribution of RBCs, suggesting the presence of autoagglutination or presence of a disproportionate number of large nucleated cells. If the latter exists, another smear should be prepared. Additionally this examination helps for rapid detection of large abnormal cells.
- *40x Examination*, using this, find an area of the smear in which the RBC are evenly distributed. Scan 8 to 10 fields in this area of the smear and determine the average number of white blood cells (WBCs) per field. Multiply the average number of WBCs per high power field by 2000 to get an approximation of the total WBC count/mm³.
- *100x Examination*, the next step in smear evaluation is to perform the WBC differential. This is done in the same area of the smear as the WBC estimate using the 100x oil immersion objective. Characteristically the differential count includes counting and classifying 100 consecutive WBCs and reporting these classes as percentages. Any WBC abnormalities are also reported. The RBC, WBC, Platelet morphology evaluation are also performed under the 100x oil immersion objective. The area of morphology is examined in a consistent scanning pattern (Figure 2-2) to avoid counting the same cells twice.

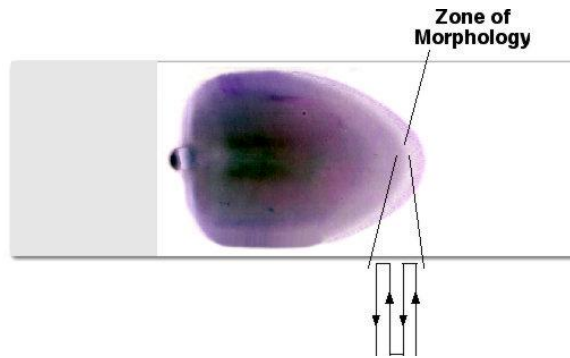


Figure 2-2 Pattern for performing a White blood Cell differential.

2.3 Leukocytes Count

A leukocytes count is almost always done as part of a complete blood count (CBC). The white blood cell (WBC) count quantifies the number of white blood cells in a sample of blood. An abnormal high or low count may suggest the presence of an illness. White blood cells are produced in the bone marrow and protect the body against infection and aid to the immune response. If there is an infection, white blood cells will attack and destroy the bacteria, fungus, or virus causing the infection. When abnormal results are obtained, it might mean the presence of a disease. There are five main types of white blood cells [20]:

- Basophils
- Eosinophils
- Lymphocytes
- Monocytes
- Neutrophils

Normal Leukocytes (WBC) count can be done through a manual method or by using automated cell counters. Automated cell counters don't include immature cells [2].

2.3.1 Automated leukocyte count

Until the 1980's, the relative number (percentage) of each type of white blood cell was determined by manual examination of the peripheral blood smear and multiplied by the white cell count to obtain the absolute leukocyte count (cells/mL). Unfortunately, the manual differential is labor intensive, subjective, statistically unreliable (only 100-200 cells are counted), and inaccurate because of nonrandom distribution of cells on the smear (monocytes at the edge, lymphocytes in the middle) [21].

Hematology analyzers are more accurate than the manual count for leukocyte elaboration under standard circumstances. For example, the hematology analyzers used in the Hematology Laboratories generate a five-part differential based on electronic impedance, conductivity, and light scatter measurements. Cell counting with these instruments is rapid, objective, statistically significant (8000 or more cells are counted), and not subject to the distributional bias of the manual count. In addition, the precision of the automated differential makes the absolute leukocyte count reliable and reproducible.

Hematology analyzers cannot yet correctly identify all abnormal white blood cells, thus; needing manual examination of the peripheral smear is still needed under some circumstances (blasts and immature cells, atypical lymphocytes, leucopenia, etc.). Specimens that meet one or

more of abnormal criteria are flagged for manual examination. Depending on the patient population, a manual smear examination is required in only approximately 25% of peripheral smears.

Current hematology analyzers cannot perform morphological analysis of cells. That is why distinct methods to automate morphological analysis are a current need.

2.3 Cells of Interest

This work focused on two cells, Metamyelocyte and Band Neutrophil which are the precursors (immature forms) of Polymorphonuclear Neutrophil and Segmented Neutrophil. Their features of the precursors are shown on Table 2-1

These cells were chosen due to our interest to contribute on the improvement of automatic analyzers as previously mentioned; current automated analyzers are not reliable at counting abnormal cells.

CELL TYPE	SIZE	NUCLEUS	CYTOPLASM	N/C RATIO	REFERENCE INTERVAL
Metamyelocyte	10-15um	Indented; kidney bean shape. Indentation is less than 50% of the width of a hypothetical round nucleus. Nucleoli: Not visible. Chromatin: Coarse clumped.	Pale blue to pink. Granules: Primary: Few. Secondary: Many (Full complement)	1.5:1	P.B.:0% B.M.:13-22%

Band	10-15um	C o S shaped. Constricted but no threadlike filament. Nucleoli: Not visible. Chromatin: Coarse lumped Note: Chromatin must be visible in constriction, may be folded over.	Pale blue to pink. Granules: Primary: Few Secondary: Abundant	Cytopla sm predomi nates	BM:17%-33% PB:0-5%
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Table 2-1 Precursors Features

From Table 2-1 we use the morphological features of nucleus; for Metamyelocyte: *nucleus has kidney or bean shape. Its indentation is less than 50% of the width of a hypothetical round nucleus.* Similarly for the Band its nucleus *has C o S shaped, constricted but no threadlike filament.*

2.4 Summary

This chapter described the concepts of importance for the development of this work such as, the manual examination of blood smears, leukocytes count and the cells' features which are the fundament for this work algorithm development.

Next chapter will describe the procedure for the design and development of the Matlab algorithm.

Chapter 3 ALGORITHM DEVELOPMENT USING MATLAB

3.1 Introduction

The algorithm was designed in MATLAB due to we can visualize results easily, and besides, it facilitates corrections in design.

During the design and development of algorithm special effort will be placed to ease the VHDL module development. A simple flow chart of the algorithm is shown below.

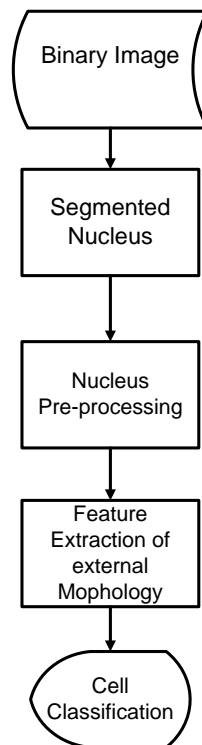
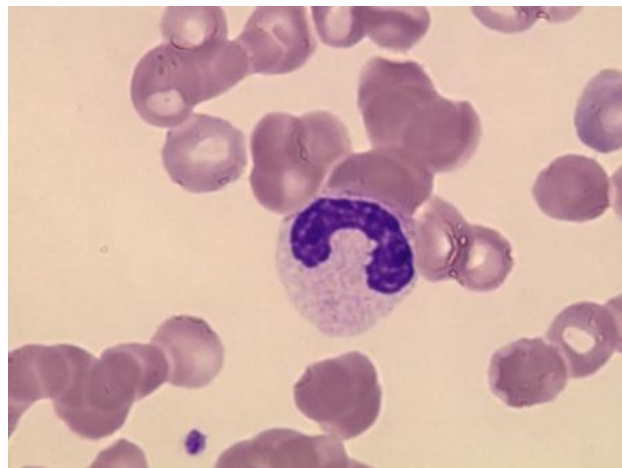


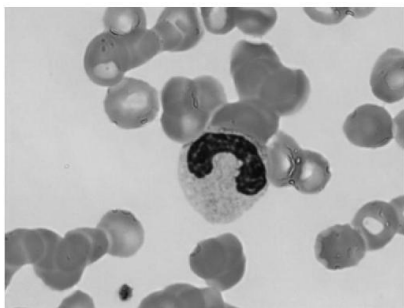
Figure 3-1 Flow Chart of algorithm

3.2 Nucleus Segmentation

Original image was binarized to separate nucleus from cytoplasm. Binarization was made using a threshold given that in this case a nucleus is darker than the cytoplasm and Red Blood Cells (RBC). From tests on sample images and based on the results it was decided to use the green plane from the RGB image for giving better segmentation by using this plane and reducing memory.



(a)



(b)



(c)

Figure 3-2 (a) Original Image, (b) Green plane Image, (b) Binary Image

The segmented nucleus was obtained from the binary image by removing the background as shown on Figure 3-2.

Our principal objective is the feature extraction of cells' nucleus.



Figure 3-3 Segmented Nucleus

3.3 Nucleus Pre-processing

Once we have a nucleus segmented, our approach reduces dimensionally to ease further image analysis.

First of all, the image was divided in windows (smaller sections), every window has only two pixels values '1' and '0', obtained by counting the nucleus pixel on the window and its percentage with a threshold to decide a '1' or '0' for each window computed. As shown in Figure 3-3.

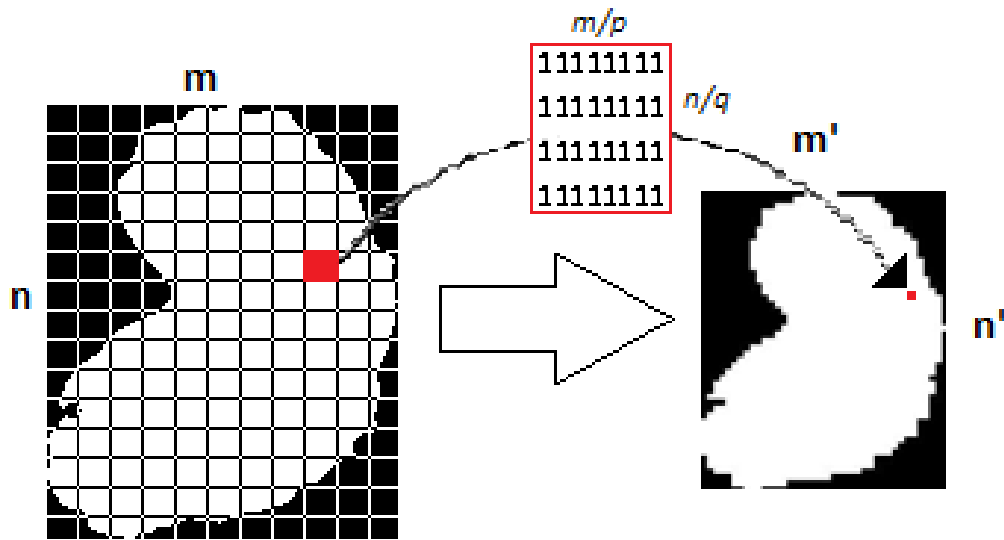


Figure 3-4 Nucleus Reduction

For each window is mapped to one pixel with value '1', if the number of 'ones' is greater than 50% of total number of pixels in the window; otherwise the pixel value is '0'.

If m/p and n/q are the dimensions for each window (Figure 3-4) we have:

$$\alpha = \frac{m}{p} * \frac{n}{q} \tag{3.1}$$

α = Total number of pixels into the window.

As we can see, the window is a matrix of α elements. Which was named X thus,

$$\beta = \sum_{i,j=1}^{m/p, n/q} x_{i,j} \tag{3.2}$$

If $\beta > 0.5\alpha$ and $y_{i,j'}$ is an element of reduced image, then:

$$y_{i,j'} = 1$$

Otherwise:

$$y_{i,j'} = 0$$

The previous process was repeated for each window in the original image and finally we got the reduced image as a matrix:

$$Y = \begin{pmatrix} y_{11} & \cdots & y_{1n'} \\ \vdots & \ddots & \vdots \\ y_{m'1} & \cdots & y_{m'n'} \end{pmatrix} \quad (3.4)$$

Given that images can be taller or wider oriented and with the purpose to ease further processing, wider images are transformer to taller images, as shown in Figure 3-5.

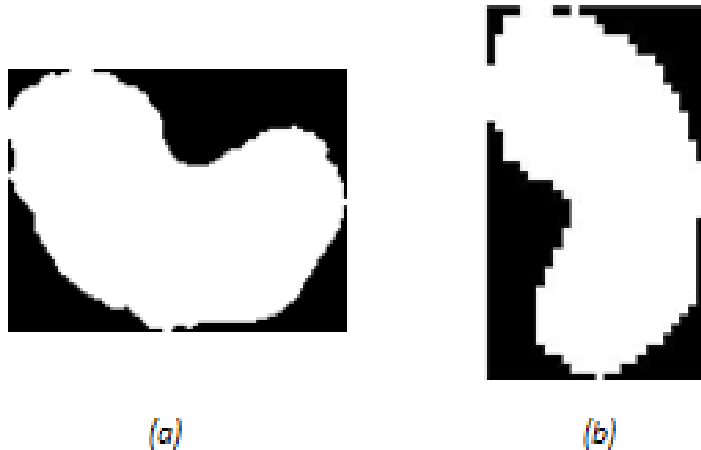


Figure 3-5 (a) Original Segmented Nucleus, (b) Reduced Vertical Nucleus

Once the nucleus was pre-processed, it can be used to extract information easily, for cells' future classification, this means that the pre-processed nucleus gave us viability for the next steps of our algorithm.

3.4 Feature extraction

We argue that by extracting zeros and ones sequences per row of the image, it can be used to extract features that define different nucleus types. Thus, extracting features of the pre-processed image was done by counting sequences of '1' (ones) and '0' (zeros) per row of the image. Figure 3-6 show this.

Every array's cell has two numbers, the pixel value is the second one and the quantity of chained pixels is the first one, for example on Figure 3-7 the row in bold has [8, 0] meaning that the pixel value is '0' in the quantity of 8 contiguous '0's. Followed by a chain of 27 '1's [27, 1] and this followed by a chain of 5 '0's.

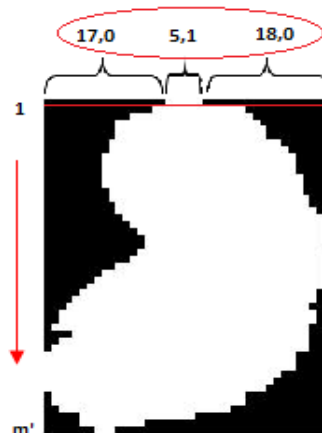


Figure 3-6 Pixels Counting

When there is a change of chain of values at the same row, the counting restarts, for example [8, 0] [27, 1] [5, 0] this means that this row had 2 changes (0 to 1 and 1 to 0).

[17,0]	[5,1]	[18,0]	[8,0]	[31,1]	[1,0]
[15,0]	[13,1]	[12,0]	[7,0]	[32,1]	[1,0]
[12,0]	[17,1]	[11,0]	[6,0]	[33,1]	[1,0]
[10,0]	[20,1]	[10,0]	[5,0]	[33,1]	[2,0]
[10,0]	[21,1]	[9,0]	[4,0]	[34,1]	[2,0]
[10,0]	[21,1]	[9,0]	[3,0]	[34,1]	[3,0]
[9,0]	[24,1]	[7,0]	[2,0]	[36,1]	[2,0]
[9,0]	[25,1]	[6,0]	[2,0]	[36,1]	[2,0]
[8,0]	[27,1]	[5,0]	[1,0]	[36,1]	[3,0]
			[4,0]	[33,1]	[3,0]
[8,0]	[27,1]	[5,0]	[2,0]	[35,1]	[3,0]
[8,0]	[27,1]	[5,0]	[1,0]	[36,1]	[3,0]
[8,0]	[28,1]	[4,0]	[35,1]	[5,0]	[]
[8,0]	[28,1]	[4,0]	[35,1]	[5,0]	[]
[9,0]	[27,1]	[4,0]	[35,1]	[5,0]	[]
[9,0]	[28,1]	[3,0]	[33,1]	[7,0]	[]
[10,0]	[27,1]	[3,0]	[32,1]	[8,0]	[]
[10,0]	[28,1]	[2,0]	[31,1]	[9,0]	[]
[11,0]	[28,1]	[1,0]	[1,0]	[27,1]	[12,0]
[12,0]	[27,1]	[1,0]	[1,0]	[27,1]	[12,0]
[13,0]	[26,1]	[1,0]	[2,0]	[24,1]	[14,0]
[14,0]	[25,1]	[1,0]	[5,0]	[16,1]	[19,0]
[14,0]	[25,1]	[1,0]	[5,0]	[9,1]	[26,0]
[13,0]	[27,1]	[]	[7,0]	[3,1]	[30,0]
[12,0]	[27,1]	[1,0]			
[10,0]	[29,1]	[1,0]			

Figure 3-7 Arrays of chains of zeros and ones per row

3.5 Recognition and Classification

This work was focused on two immature WBC, Metamyelocyte and Band Neutrophil, whose features were described on *chapter 2*.

For Metamyelocytes' recognition, it is sufficient to identify the indentation on their nucleus because it is its unique morphological feature [3].

Given that we have always taller images and knowing that Metamyelocytes have the indentation on the central part of nucleus, we can reduce the number of rows to explore, by limiting to the central portion of the rows of the matrix.

Similarly, Band Neutrophils' recognition can be done by identifying the width of their nucleus, since this cell has a thin nucleus with S or C form [3]. This could be performed by getting the proportions of the nucleus with the background of the pre-processed nucleus.

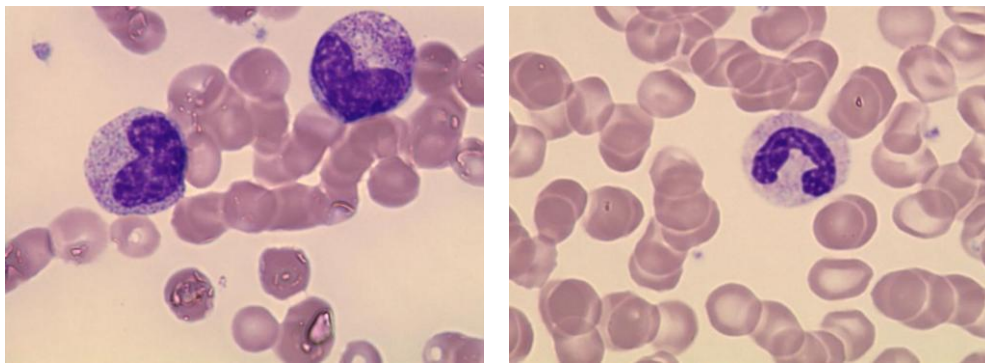


Figure 3-8 (a) Metamyelocyte, (b) Band Neutrophil

Once the array of portions is obtained, we can focus on the design rules for the classification of each cell.

3.5.1 Band Neutrophil Recognition

For Recognition and classification of Band Neutrophil, we followed some steps, as shown on Figure 3-9. These steps were decided based on a previous study of features of this cell type.

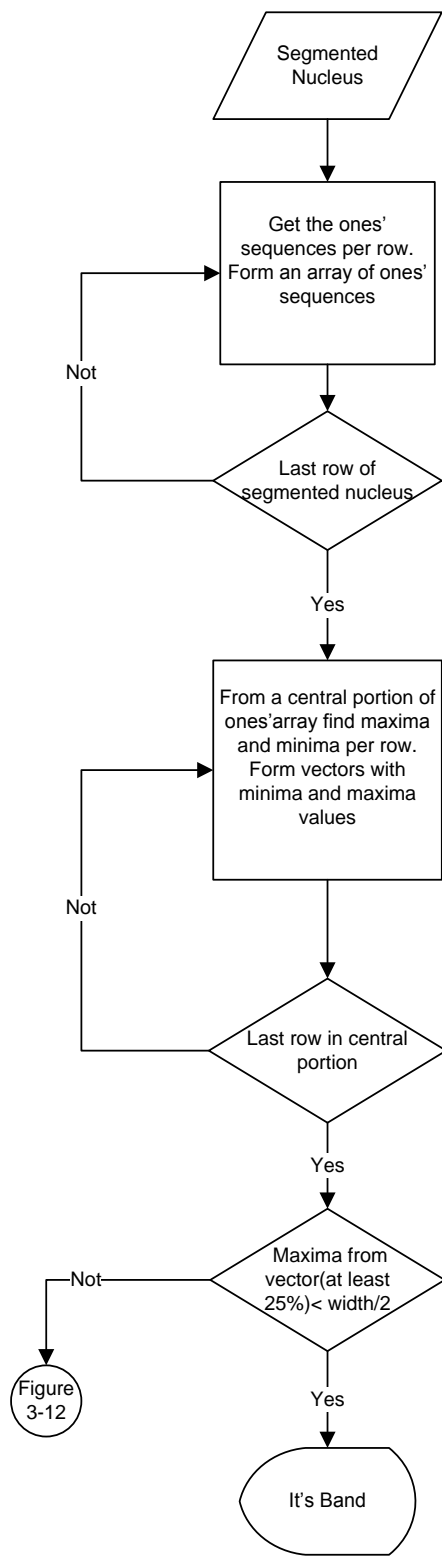


Figure 3-9 Band Netrophil Recognition and classification

- Firstly we get the ones' quantities from array in order to form a new one, which contains only the sequences of ones. See Figure 3-10. On this figure we can notice that there is just as single sequence of ones. It is expected but not necessary that Band Neutrophil could have more than one sequence of ones in a row.

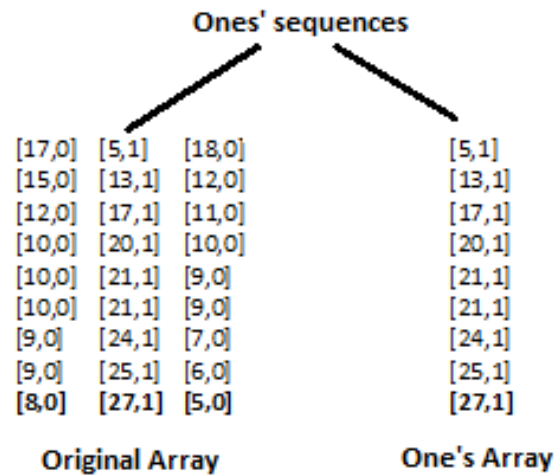


Figure 3-10 Ones' Array Construction

We extracted the minimum and maximum values of ones in the array per row and save these values in a column vector for each. These values helped to make a decision. If we find a considerable amount of maxima in vector (25% of vector, approximately) which were less than the half of image's width the processed cell is classify as Band Neutrophil. The minima values were discarded when these were less than 10%.

Finally the recognition and classification of the Band Neutrophil was made when maxima in the vector has to be less than half of image's width, if processing cell has this feature is a Band Neutrophil.

3.5.2 Metamyelocyte Recognition

As was previously reported Metamyelocyte is the youngest Neutrophil generally reported. The nucleus is *indented*, and is usually “bean or Kidney”-shaped.

As well as Band Neutrophil we have some steps (See Figure 3-12) for its recognition and classification of this cell. In figure 3-9 we can notice that once the process of recognition a Band was done and the cell wasn't recognized as a band, we proceed to check if the cell is a Metamyelocyte.

- From the original array we extract the zeros' sequences. First from left to right and first from right to left per row in order to form an array with two columns with zeros. As shown on Figure 3-11.

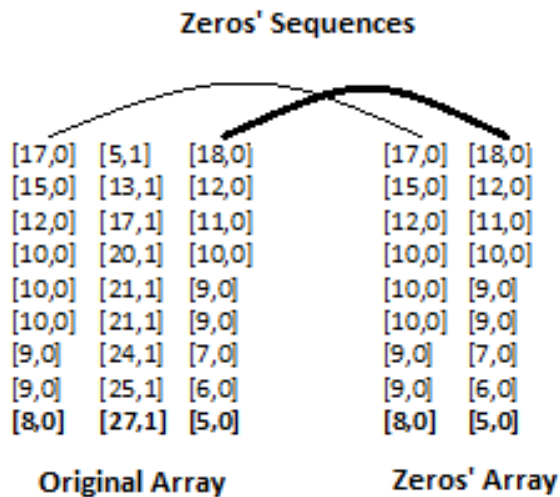


Figure 3-11 Zeros' Array Construction

- Once the array is formed, a trial vector of smaller size array (25%) was used for finding a change in the array, such as we localized a maximum into the trial vector, we check all values before in order to identify the existence of rising tendency and the values after for identify an downward tendency.
- If these tendencies are founded, the trial vector values were verified to be less than the half width of nucleus' image. If we have these tendencies just in one column or side of nucleus, is a Metamyelocyte due to it has only one dent on the longer part of its nucleus. When there are two dents, and the minimum is bigger than 10% of width of segmented nucleus is a Band otherwise is indeterminate.

As well as Band, Metamyelocyte has to carry out with the features described before. Due to we are looking for a dent we have to find tendencies that give us reference about nucleus' form.

When cell is neither Band nor Metamyelocyte we classify it as "*indeterminate*".

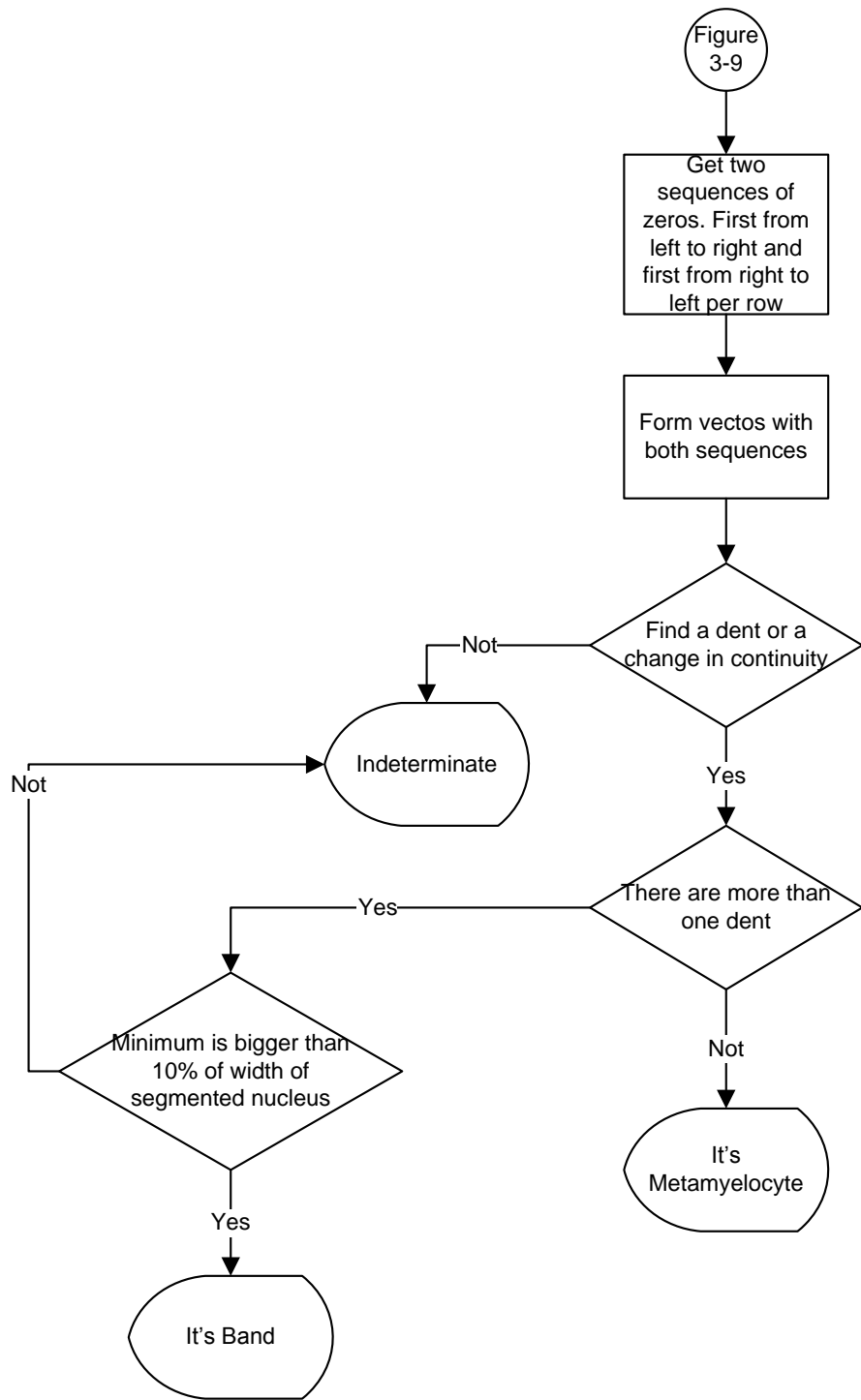


Figure 3-12 Metamyelocyte Recognition and classification

3.6 Summary

On this chapter, the designed algorithm in MATLAB was described. This Chapter was divided in four main parts: Nucleus segmentation, Nucleus pre-processing, Feature extraction, and Recognition and classification.

Next chapter shows how some VHDL entities were developed from the presented algorithm in MATLAB.

Chapter 4 VHDL IMPLEMENTATION

4.1 Introduction

After completing the MATLAB version, we designed and developed enough modules (Entities) in VHDL to show applications that classify two different abnormal white blood cells from peripheral blood samples.

The modules were designed and synthesized with the software Xilinx ISE 12.1; Figure 4-1 shows the workspace for the software used. At the same way these were tested and proof on a FPGA Spartan-3E 500K, using a Nexys 2 Board.

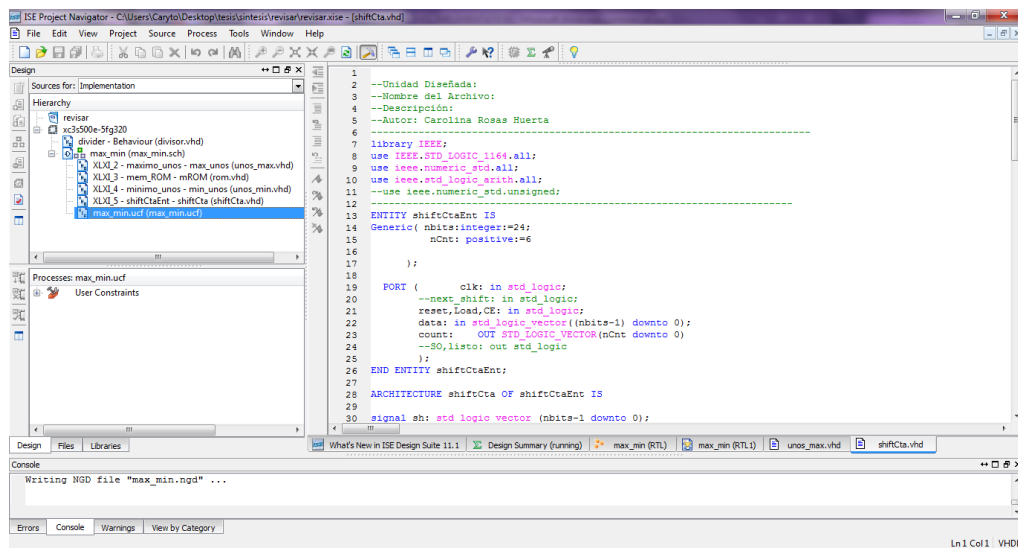


Figure 4-1 Workspace ISE Xilinx.

4.2 VHDL Algorithm Implementation

VHDL Implementation was done using the MATLAB algorithm as base; entities were designed for the main part of this algorithm in order to show that is possible to develop the rest of the modules, as well as, new ones.

For the rest part, the tests were carry out by hand. The designed system is shown in Figure 4-2.

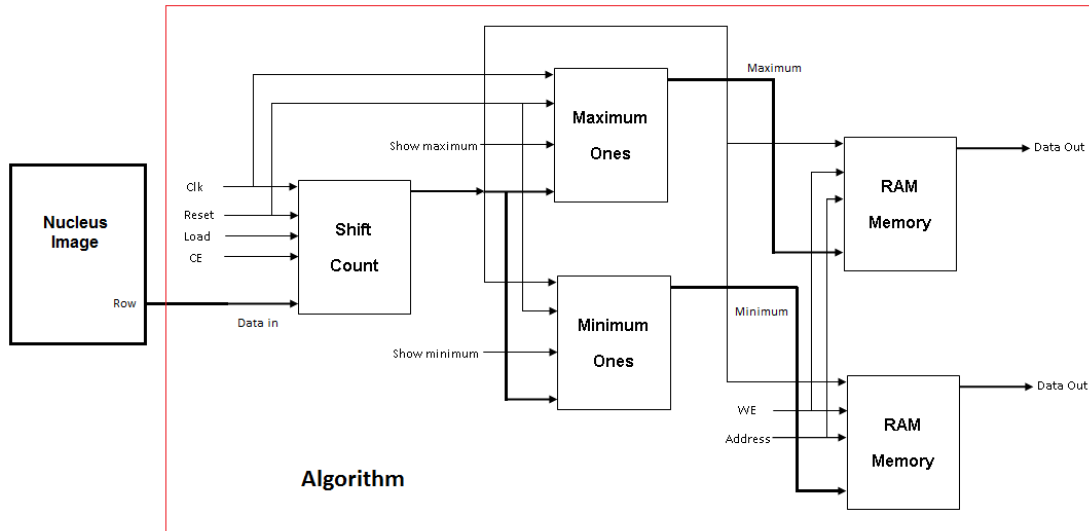


Figure 4-2 VHDL System Designed

The system was synthesized and the RTL schematic is shown below in Figure 4-3.

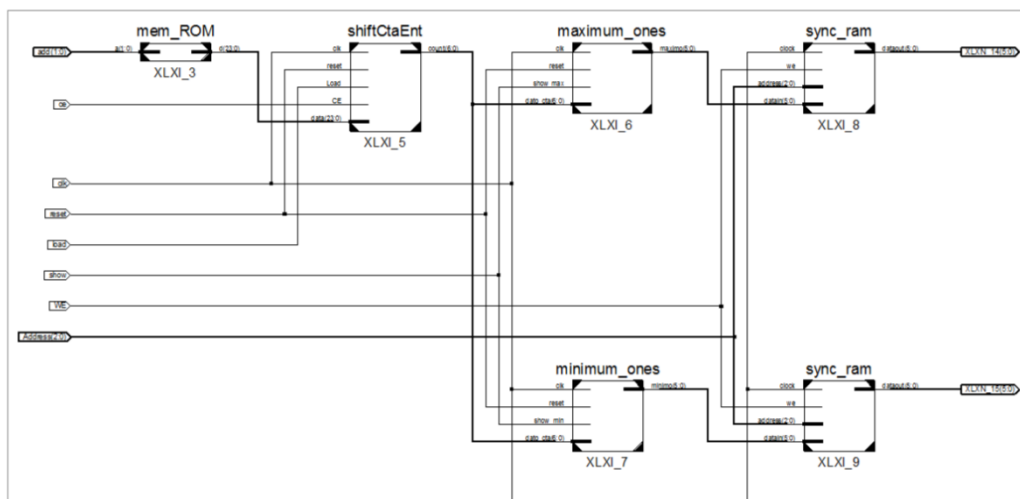


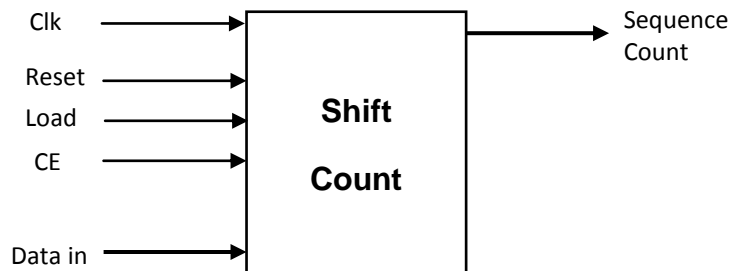
Figure 4-3 RTL schematic for system

4.3 Design Entities Description

The description for each entity designed is written below; this description has the list of components (signals), its purpose, and synthesized RTL Schematic for each module

4.3.1 Count and Shift Entity

This entity has the purpose of getting row by row from image and it counts the sequences of ones and zeros.



Element	Description
Clk	Clock Signal
Reset	Reset Signal
Load	Load the shift register
CE	Chip Enable
Data in	Input Data Bus
Data out	Output Data Bus

This entity performs the main part of algorithm due to our algorithm is based on the count of sequences of ones and zeros, this entity receive data from a memory, this entity has two parts a shift register and a module of count.

The shift register has parallel load and serial output, the output is connect to the count part; this count one by one the pixels give by the shift, such if exists a change in the sequences from 1 to 0 or 0 to 1, this part give as a output a vector form by 7 bits, 6 bits for the count and one for the pixel value (0 or 1) as shown on Figure 4-4.

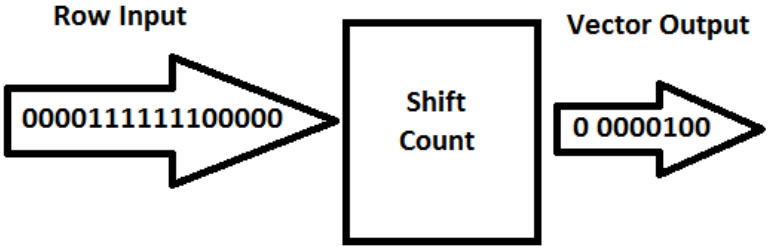


Figure 4-4 Input/Output example for Shift Count Entity

Figure 4-5 shows the RTL Schematic synthesized with the Xilinx ISE 12.1 software for the Shift and Count module.

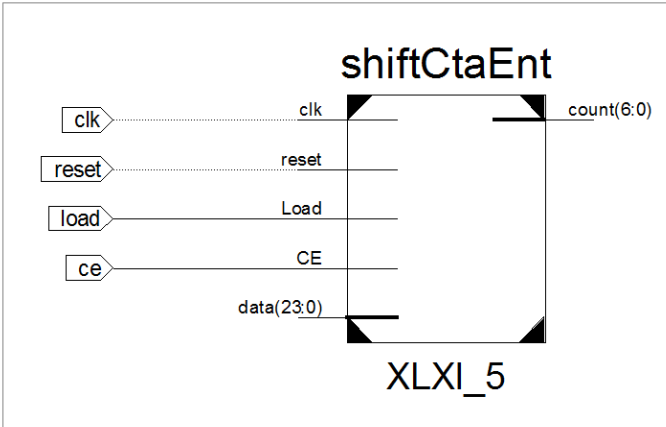
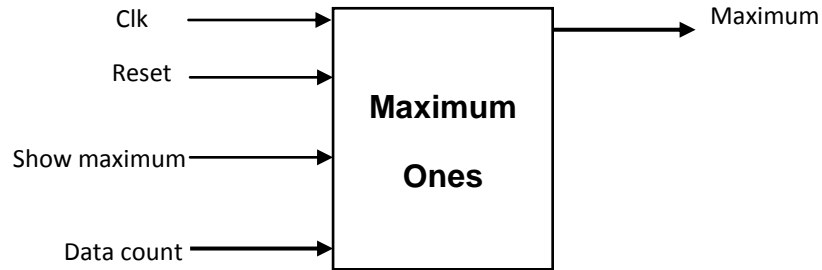


Figure 4-5 Entity Shift and Count RTL

4.3.2 Maximum Ones Entity

This entity has the purpose of find the maximum value of ones, from given sequences by *Count and Shift Entity*.



Element	Description
Clk	Clock Signal
Reset	Reset Signal
Show Maximum	Enable the output
Data count	Input Data Bus from Shift and Count Entity
Maximum	Maximum Output

This module has as input the sequences from previous entity, if the module detect a sequence of one, this sequence is compared with a register; if the value is bigger than the value in the register this value is replaced by the sequence, this process is repeated until the row are finished. Once the last sequence is processed the module gives an output with the maximum value of row.

Figure 4-6 shows the RTL Schematic synthesized with the Xilinx ISE 12.1 software for the maximum ones entity.

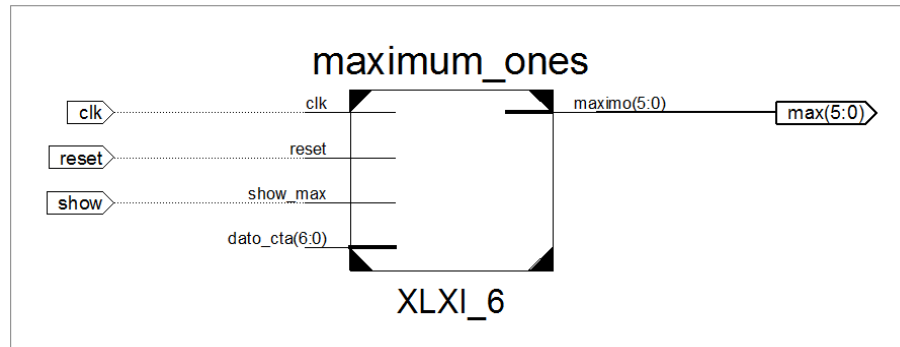
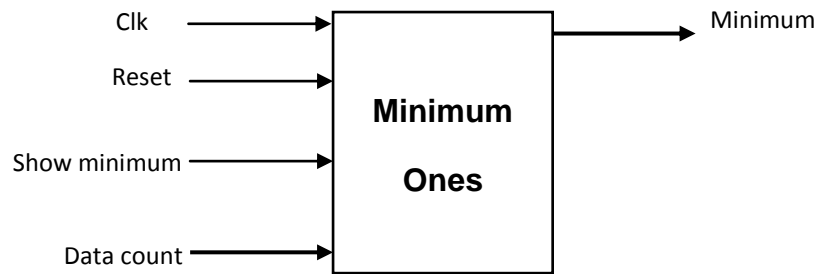


Figure 4-6 Entity Maximum ones RTL.

4.3.3 Minimum Ones Entity

This entity has the purpose of find the minimum value of ones, from given sequences by *Count and Shift Entity*.



Element	Description
Clk	Clock Signal
Reset	Reset Signal
Show Minimum	Enable the output
Data count	Input Data Bus from Shift and Count Entity
Minimum	Minimum Output

This module has as a input the sequences from *Count and Shift Entity*, if the module detect a sequence of one, this sequence is

compared with a register; if the value is smaller than the value in the register this value is replaced by the sequence, this process is repeated until the row are finished. Once the last sequence is processed the module gives an output with the minimum value of the row.

Figure 4-7 shows the RTL Schematic synthesized with the Xilinx ISE 12.1 software for the maximum ones entity.

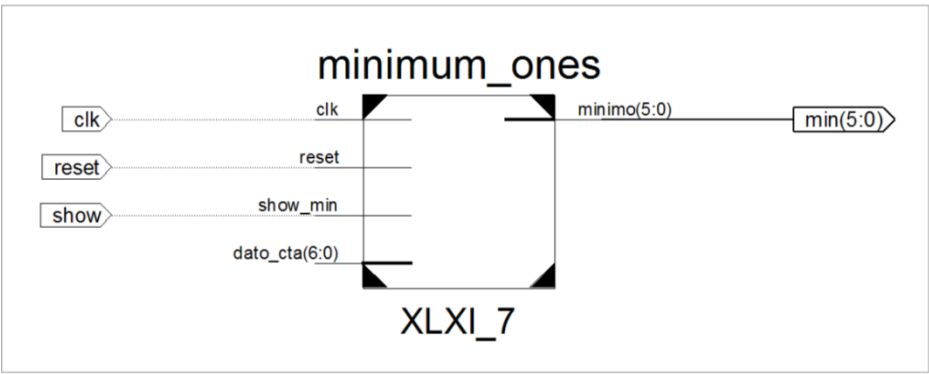
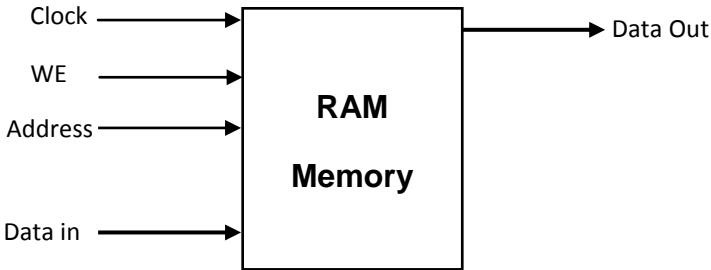


Figure 4-7 Entity Minimum ones RTL.

4.3.4 RAM Memory

This entity has the purpose of save the maxima and minima values per row.



Element	Description
Clock	Clock Signal
WE	Write Enable
Address	Read/ Write Address
Data in	Data input
Data out	Data Output

This module has two inputs; first is the data bus from *Minimum ones* or *Maximum ones entities* and the address, which is the same as the row of processed image. This module is used to save the maxima and minima value of each row in order to form and a array with these values.

Figure 4-8 shows the RTL Schematic synthesized with the Xilinx ISE 12.1 software for the Ram memory entity.

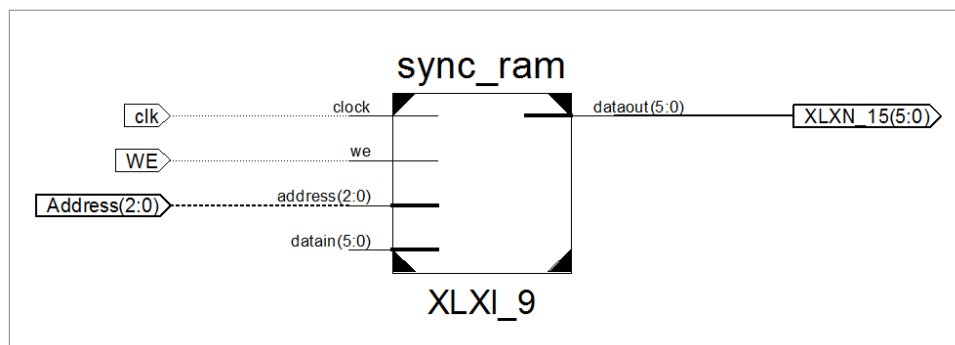


Figure 4-8 Entity RAM Memory RTL.

4.4 Summary

On this chapter the VHDL implementation was explained, also, we described the performance of each module.

Designed modules open the possibility to develop *detection, recognition* and *classification* of other abnormal blood cells by defining different module interconnections and possibly other new modules.

Next chapter shows the results for the MATLAB algorithm and VHDL implementation.

Chapter 5 TESTS AND RESULTS

5.1 Introduction

This section shows some results obtained for this work: Algorithm development in MATLAB and VHDL implementation. A set of RGB images was used for testing the MATLAB algorithm. Special care was taken to obtain images from universities [22], lab sites [23], and from atlases [24], [25]. This ensures that the selected images were classified by experts to be either Metamyelocytes or Band Neutrophils.

As mentioned on Chapter 3, the set of images were pre-processed. Single channel images provide visually useful results for our problem domain.

5.2 MATLAB Algorithm Results

The Algorithm development in MATLAB helps us to visualize results easily, and besides, it eases corrections in design.

The output gives us a message which indicates the cell type, such as, “It’s Band”, “It’s Metamyelocyte” and “Indeterminate”

We use RGB images with different sizes, this is possible given that the algorithm uses only morphological features of the nucleus and this is segmented and reduced for testing the algorithm.

The algorithm firstly checks if the cell is a Neutrophil Band; if it is not then, checks if it is a Metamyelocyte, when the algorithm does not identify the features it displays as “Indeterminate”; Table 5-1 shows sample messages as as they will be displayed for each type.




Nucleus Image	Cell type	Message
	Band Neutrophil	"It's Band"
	Metamyelocyte	"It's Metamyelocyte"
	Myelocyte	"Indeterminate"

Table 5-1 Algorithm Message Output.

As previously mentioned before, the algorithm processes and classifies the segmented nucleus.

In order to have a short description of how the results were obtained we will report one example for each cell mentioned on Table 5-1.

In Band Neutrophil case the following results are obtained:

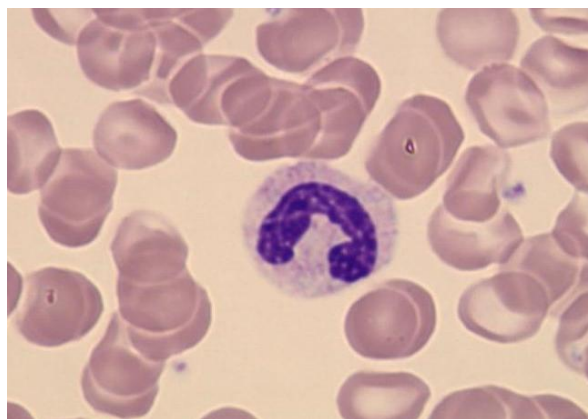


Figure 5-1 Band Neutrophil Image

From Blood Smear Image (Figure 5-1) the band nucleus is shown below on Figure 5-2. This image was binarized and segmented.



Figure 5-2 Segmented Nucleus (Band Neutrophil)

Once the nucleus was segmented, and processed, MATLAB displays an image as shown on Figure 5-3. The image has a different orientation because of the *nucleus pre-processing* described on chapter 3.

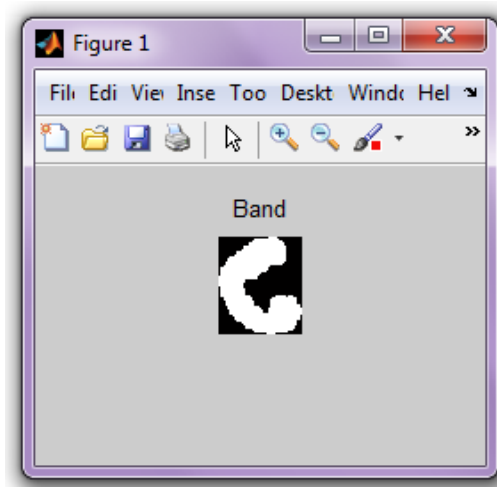


Figure 5-3 MATLAB Algorithm Result for Band Neutrophil

In addition with the previous image the algorithm shows a message in the MATLAB workspace; "*It's Band...Bandbw2.bmp*". The features for the correct classification are described on table 5-2.

Features	Values
Maximum Number of changes	3
Minima and Maxima	$< \frac{Width}{2}$
Dent Vector	Not found

Table 5-2 Considered features for Band classification

From table 5-2 data we can verify that for the test image is classified as Band and is correct to classify it based on the number of changes in the sequences, and that minima and maxima values were less than $< \frac{Width}{2}$.

For a Metamyelocyte example; the following results were obtained:

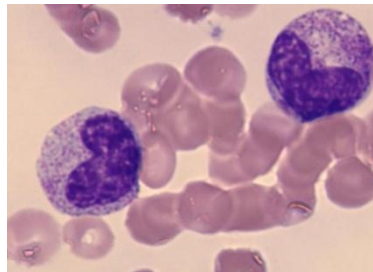


Figure 5-4 Metamyelocyte Image

Figure 5-4 shows a Blood Smear Image, corresponding segmented nucleus for one of these cells is shown below on Figure 5-5.



Figure 5-5 Segmented Nucleus (Metamyelocyte)

Nucleus of figure 5-5 is processed and the result from algorithm is shown on figure 5-6, this image has the reduced nucleus and a title with the type of cell.

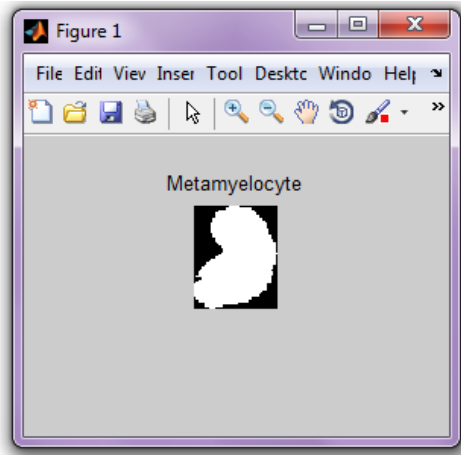


Figure 5-6 MATLAB Algorithm Result for Metamyelocyte

As well as in Band Netrophil classification, the algorithm shows a message on the workspace, in this case this message is “*It 's Metamyelocyte...metabw1.bmp*”.

For this example the algorithm classifies the nucleus finding one vector with the tendencies mentioned on chapter 3, together with the number of changes and the minima and maxima per row was verified, and shown on Table 5-2.

Features	Values
Maximum Number of changes	1
Minima and Maxima	$> \frac{Width}{2}$
Dent Vector	10 10 11 12 13 14

	14
	13
	12
	10

Table 5-3 Considered features for Metamyelocyte classification

From table 5-3 we can notice that the algorithm classifies the cell as a Metamyelocyte due that:

- only 1 change in sequences exists,
- that minima and maxima $> \frac{Width}{2}$ and
- that a dent vector exists

The third case is when the cell is neither Band nor Metamyelocyte; for example, a Myelocyte (Figure 5-7).

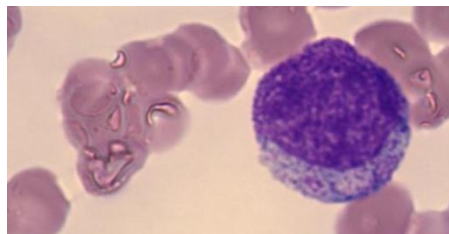


Figure 5-7 Myelocyte Image

In this case the algorithm classifies the cell as “*indeterminate*” because the algorithm does not recognize this cell type.



Figure 5-8 Segmented Nucleus (Myelocyte)

From the segmented nucleus we obtain the following data on table 5-4, which shows that the cell does not comply with the known features.

Features	Values
Maximum Number of changes	1
Minima and Maxima	$> \frac{Width}{2}$
Dent Vector	Not found

Table 5-4 Considered features for classification

5.3 VHDL Implementation Results

As was explained on Chapter 4, modules were designed and synthesized with the software Xilinx ISE 12.1. And tested in a FPGA 3E 500K; results from the implemented modules will be illustrated below

5.3.1 Synthesis Results

In this part we report the results for the synthesis, such as the RTL Schematics and the synthesis reports. This information was obtained from the Software before mentioned.

The following Figures show the RTL schematic for every designed module. These schematics display the internal elements synthesized by software. Figure 5-9 show the complete RTL schematic for system shown on Figure 4-3

On Figure 5-10 the RTL Schematic for Shift and Count Entity is shown.

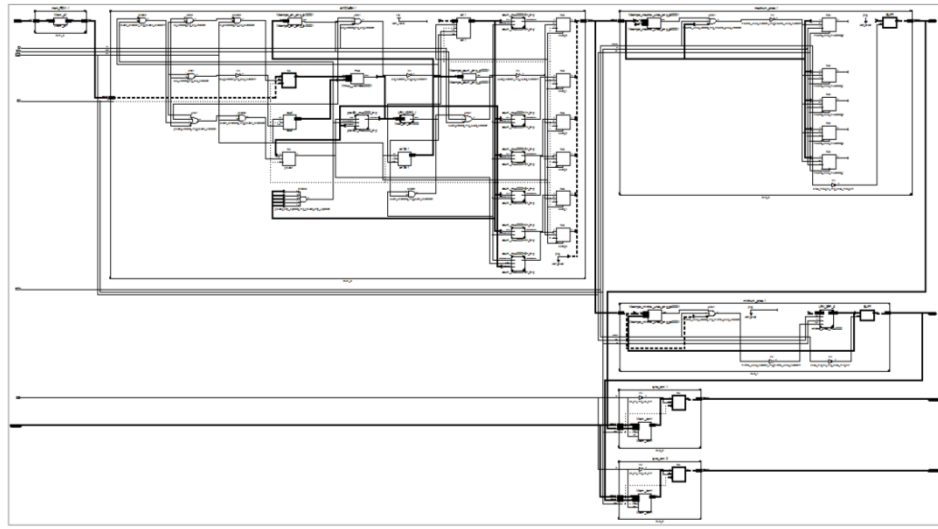


Figure 5-9 RTL schematic for complete system

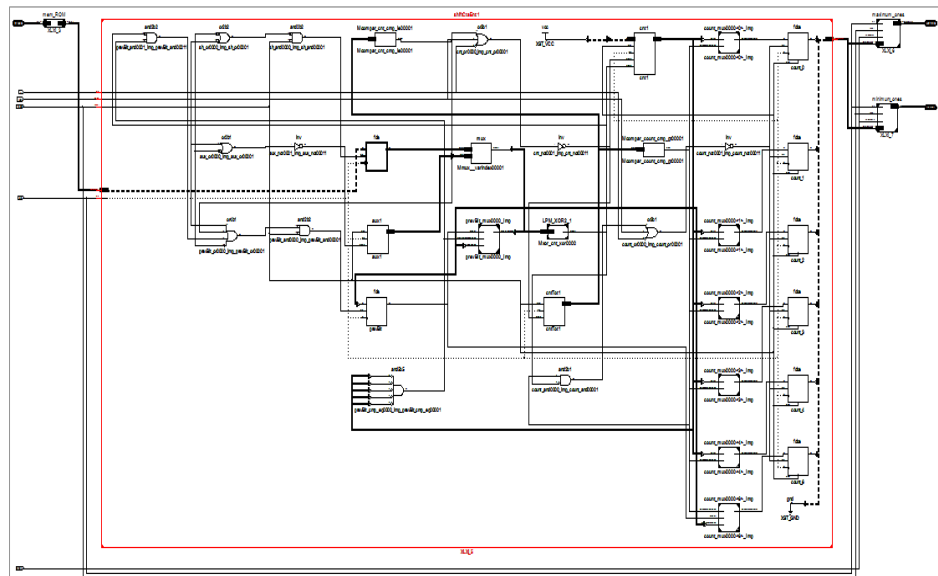


Figure 5-10 RTL schematic for Count and Shift entity

Figure 5-11 shows the RTL Schematic for the Maximum Ones Entity.

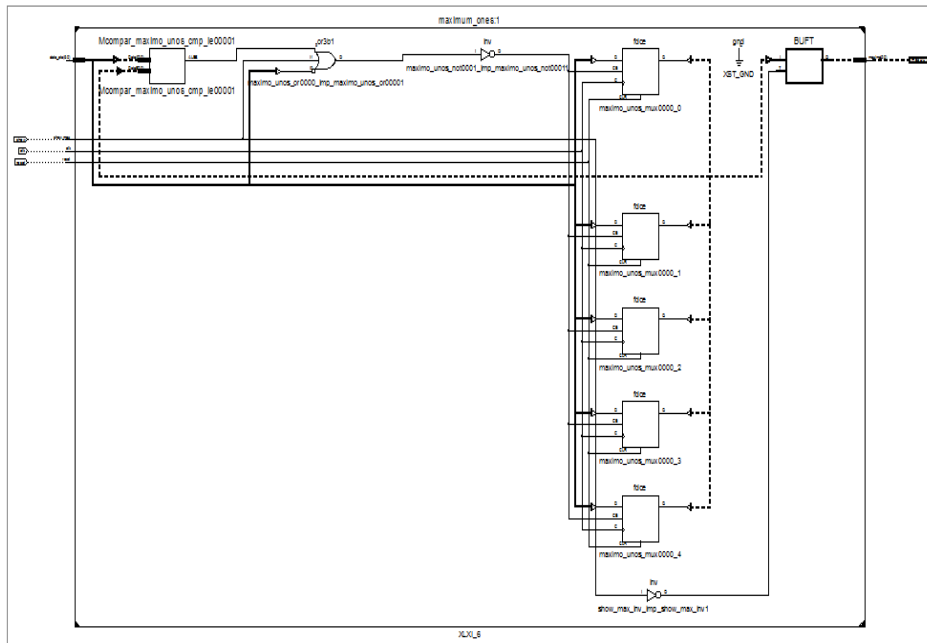


Figure 5-11 RTL Schematic for Maximum Ones Entity

Finally Figure 5-12 and Figure 5-13 show the RTL schematics for Minimum Ones and RAM Memory.

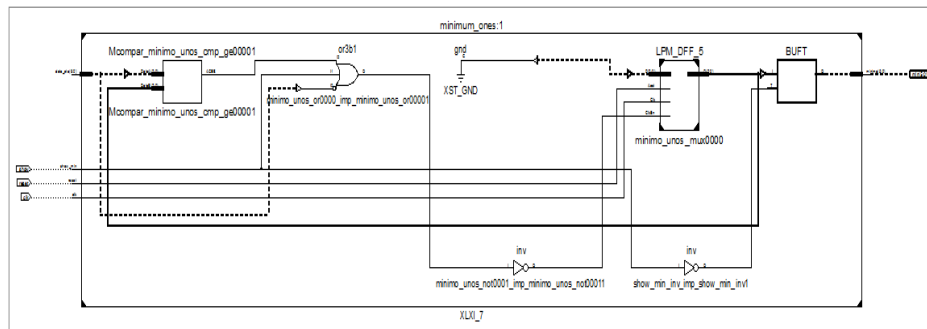


Figure 5-12 RTL Schematic for Minimum Ones Entity

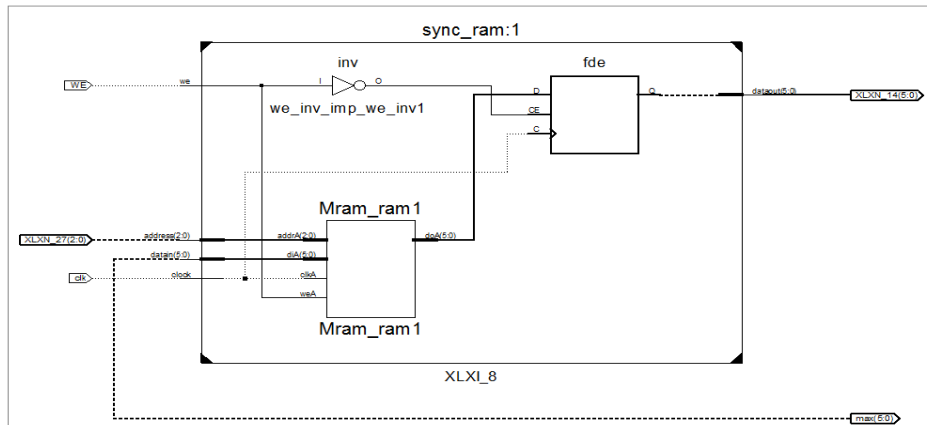


Figure 5-13 RTL Schematic for RAM Memory Entity

Table 5-5 is the cell usage report of Final results.

<i>Final Results</i>		

RTL Top Level Output File Name :	max_min.ngf	
Top Level Output File Name :	max_min	
Output Format :	NGC	
Optimization Goal :	Speed	
Keep Hierarchy :	NO	
<i>Design Statistics</i>		

# IOs :	35	
<i>Cell Usage :</i>		

# BELS :	115	
#	GND :	1
#	INV :	6
#	LUT1 :	4
#	LUT2 :	32
#	LUT2_L :	2
#	LUT3 :	15
#	LUT4 :	23
#	LUT4_D :	2
#	LUT4_L :	1
#	MUXCY :	13
#	MUXF5 :	11
#	MUXF6 :	3
#	MUXF7 :	1
#	VCC :	1
# FlipFlops/Latches :	51	
#	FDCE :	22
#	FDE :	20

#	FDPE :	9
#	RAMS :	11
#	RAM16X1S :	11
#	Clock Buffers :	1
#	BUFGP :	1
#	IO Buffers :	34
#	IBUF :	10
#	OBUF :	12
#	OBUFT :	12

Table 5-5 Synthesis Final Report

Table 5-6 shows the Device Utilization Summary

Logic Utilization	Used	Available	Utilization
Number of Slice Flip Flops	51	9,312	1%
Number of 4 input LUTs	87	9,312	1%
Number of occupied Slices	58	4,656	1%
Number of Slices containing only related logic	58	58	100%
Number of Slices containing unrelated logic	0	58	0%
Total Number of 4 input LUTs	92	9,312	1%
Number used as logic	76		
Number used as a route-thru	5		
Number used as 16x1 RAMs	11		
Number of bonded IOBs	35	232	15%
Number of BUFGMUXs	1	24	4%
Average Fanout of Non-Clock Nets	3.91		

Table 5-6 Device Utilization Summary

The Timing Summary report is reproduced as follows:

Speed Grade: -5

Minimum period: 6.311ns (Maximum Frequency: 158.441MHz)

Minimum input arrival time before clock: 5.034ns

Maximum output required time after clock: 4.134ns

Maximum combinational path delay: 6.558ns

Timing Detail:

All values displayed in nanoseconds (ns)

5.3.2 FPGA Programming Results

Tests were done using switches and led's modules available on the Nexys 2 Board, an image of it is shown in Figure 5-14.



Figure 5-14 Nexys 2 Board

Maxima and minima per row are displayed on the led's. We use three rows as an example of how were visualized the results for minimum and maximum.

(a) "00111110110000000001110" => Row of image,

This row has 3 sequences of ones; going left to right, the first has 5 ones, the second 2 and the third 3. Thus, the maximum of the row is 5 and minimum is 2. We read the memory of minimum and maximum for this row and the result was:



Figure 5-15 (a) Maximum and Minimum display on the leds

The left hand LED module indicates the maximum and the second one is the minimum.

(b) For the row with chain of bits, "0000001111110011111111", the minimum is 6 and maximum is 9, the FPGA implementation gives the result as shown on Figure 5-15 b;

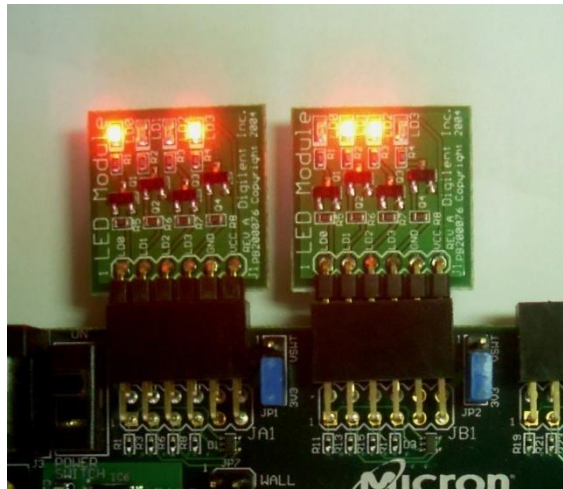


Figure 5-15 (b) Maximum and Minimum display on led's

(c) Another row with bits, "111111111111000011111111", minimum is 8 and maximum is 12, the FPGA result was as shown on Figure 5-15 (c);

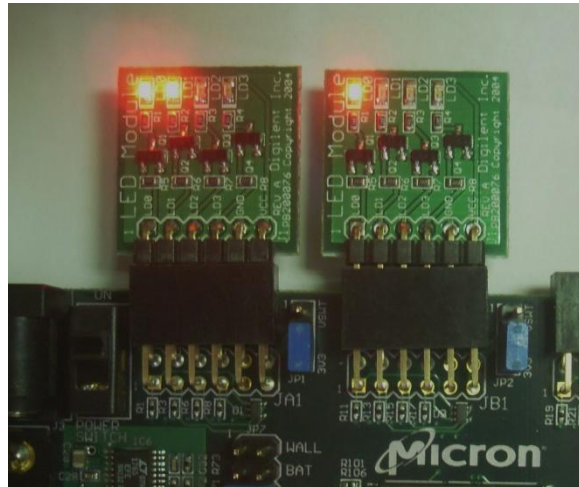


Figure 5-15 (c) Maximum and Minimum display on led's

Switches were used for handled the control signals, such as the reset, the address for the memory and the LED display control.

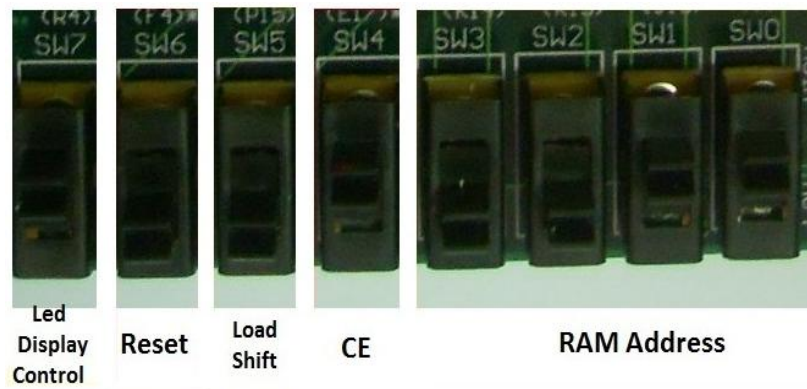


Figure 5-16 Nexys 2 Board Switches

5.4 Summary

On this chapter some results were shown for MATLAB algorithm and VHDL implementation. Table 5-7 shows the results for different Metamyelocyte and Band classification images tested.

Cell	Number of Images	MATLAB	VHDL
<i>Metamyelocyte</i>	10	100%	100%
<i>Band Neutrophil</i>	20	100%	100%

Table 5-7 Results Summary for Correct Classification

From Table 5-7 we have that for Metamyelocyte we have 10 samples and the algorithm classify correctly the 100%. For Band we have 20 samples and the recognition of the algorithm was of 100%.

Chapter 6 CONCLUSIONS AND FUTURE WORK

6.1 Conclusions

A novel algorithm for detection and recognition of Metamyelocyte and Band Neutrophil was designed and implemented in MATLAB.

The algorithm was based on nucleus' morphological features, such as indentation for Metamyelocyte and thin nucleus with S or C form for Band Neutrophil.

The test set of images were previously classified by specialists, and as a result the algorithm shows the cell's type for every image (Metamyelocyte or Band).

Hardware modules were designed and implemented as VHDL entities for the core part of the MATLAB algorithm. Using them to show applications that classify two different immature white blood cells from peripheral blood samples were built. Applications were synthesized and tested on a NEXYS II FPGA board from Digilent.

The set of modules opens the possibility to develop applications for *detection*, *recognition* and *classification* of other abnormal or immature blood cells by defining different module interconnections and possibly other new modules.

The algorithm presented for the recognition of immature forms on peripheral blood can be used as a reference for future works on this area; as well as development and implementation could contributed to improve some devices, like blood analyzers based on microscopy augmented images.

6.2 Future Work

Develop algorithms or modify our work in order to extract characteristics of cytoplasm for other classifications. These could include color and texture.

Design more modules for the identification of other immature or abnormal cells and integrate them with ours, in order to have a more immature cells classified by the system.

FIGURES LIST

Figure 1-1 MATLAB Algorithm development process.....	7
Figure 1-2 VHDL Algorithm development process.....	7
Figure 2-1 Peripheral Smear Preparation.....	10
Figure 2-2 Pattern for performing a White blood Cell differential	13
Figure 3-1 Flow Chart of algorithm.....	17
Figure 3-2 (a) Original Image, (b) Green plane Image, (b) Binary Image.....	18
Figure 3-3 Segmented Nucleus.....	19
Figure 3-4 Nucleus Reduction.....	20
Figure 3-5 (a) Original Segmented Nucleus, (b) Reduced Vertical Nucleus..	21
Figure 3-6 Pixels Counting	22
Figure 3-7 Arrays of chains of zeros and ones per row.....	23
Figure 3-8 (a) Metamyelocyte, (b) Band Netrophil	24
Figure 3-9 Band Netrophil Recognition and classification.....	25
Figure 3-10 Ones' Array Construction	26
Figure 3-11 Zeros' Array Construction.....	27
Figure 3-12 Metamyelocyte Recognition and classification	29
Figure 4-1 Workspace ISE Xilinx.....	31
Figure 4-2 VHDL System Designed	32
Figure 4-3 RTL schematic for system.....	32
Figure 4-4 Input/Output example for Shift Count Entity	34
Figure 4-5 Entity Shift and Count RTL.....	34

Figure 4-6 Entity Maximum ones RTL.....	36
Figure 4-7 Entity Minimum ones RTL.....	37
Figure 4-8 Entity RAM Memory RTL.....	38
Figure 5-1 Band Neutrophil Image	41
Figure 5-2 Segmented Nucleus (Band Neutrophil)	42
Figure 5-3 MATLAB Algorithm Result for Band Neutrophil.....	42
Figure 5-4 Metamyelocyte Image	43
Figure 5-5 Segmented Nucleus (Metamyelocyte).....	43
Figure 5-6 MATLAB Algorithm Result for Metamyelocyte.....	44
Figure 5-7 Myelocyte Image.....	45
Figure 5-8 Segmented Nucleus (Myelocyte).....	45
Figure 5-9 RTL schematic for complete system.....	47
Figure 5-10 RTL schematic for Count and Shift entity.....	47
Figure 5-11 RTL Schematic for Maximum Ones Entity.....	48
Figure 5-12 RTL Schematic for Minimum Ones Entity.....	48
Figure 5-13 RTL Schematic for RAM Memory Entity.....	49
Figure 5-14 Nexys 2 Board.....	51
Figure 5-15 (a) Maximum and Minimum display on the led's.....	52
Figure 5-15 (b) Maximum and Minimum display on led's.....	52
Figure 5-15 (c) Maximum and Minimum display on led's.....	53
Figure 5-16 Nexys 2 Board Switches.....	53
Figure A-1 Histograms and printout generated by the Coulter STKR automated hematology analyzer.....	61

Figure A-2 Run Screen generated by the *CELL-DYN® 3700* automated hematology analyzer.....62

Figure A-3 Run Screen generated by the *SYSMEX XE 2100* automated hematology analyzer.....63

TABLES LIST

Table 1-1 Summary of Previous works.....3

Table 2-1 Precursors Features.....16

Table 5-1 Algorithm Message Output.....41

Table 5-2 Considered features for Band classification43

Table 5-3 Considered features for Metamyelocyte classification.....44

Table 5-4 Considered features for classification.....46

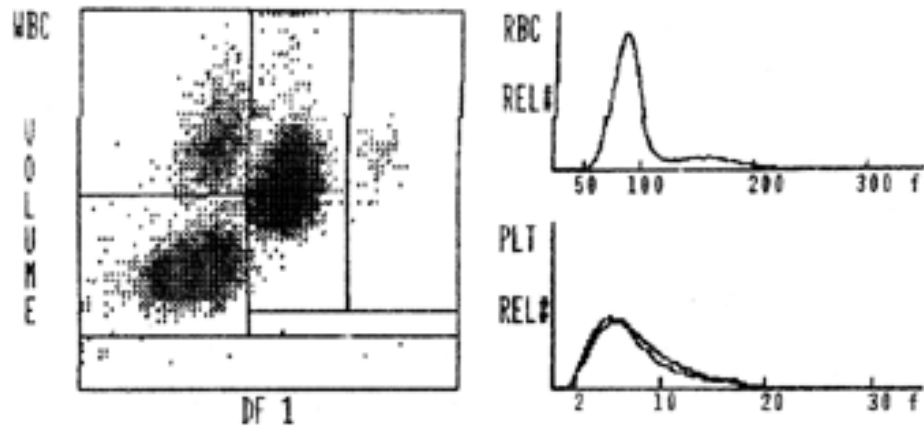
Table 5-5 Synthesis Final Report.....49

Table 5-6 Device Utilization Summary.....50

Table 5-7 Results Summary for Correct Classification.....54

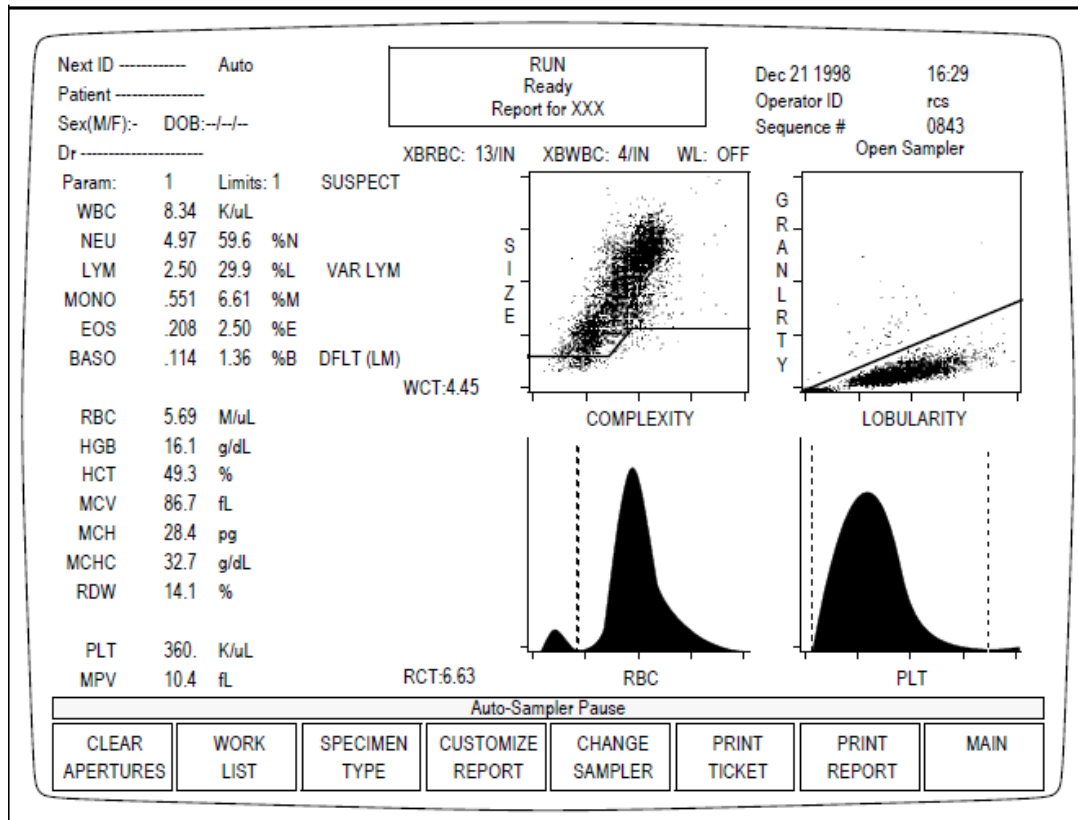
Appendix A

A.1 Results displayed by 3 Automated Blood Counting Instruments

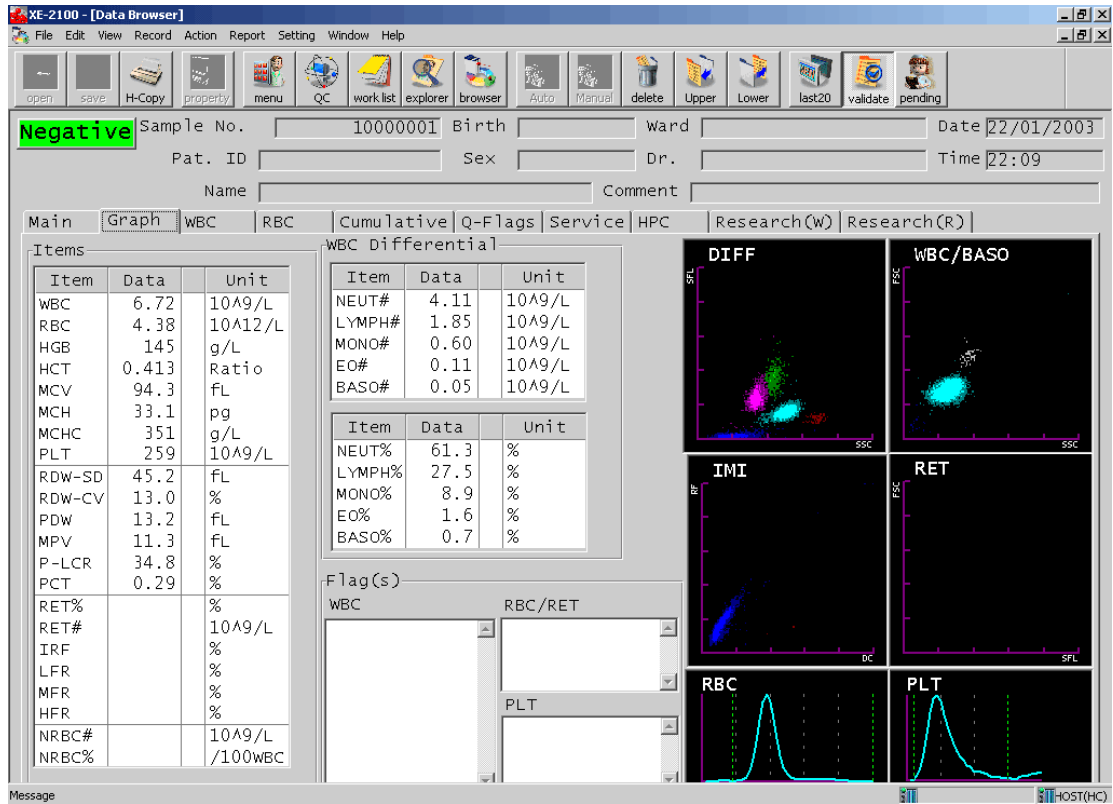


ID# 1	WBC	6.7		RBC	4.56
ID# 2		%	#	HGB	13.5
Sequence #	NE	59.4	4.1	HCT	40.3
	LY	31.6	2.1	MCV	88.3
DATE: 06/21/96	MO	7.7	0.5	MCH	29.5
TIME: 08:55:45	EO	0.7	0.0	MCHC	33.5
Cass/Pos S	BA	0.6	0.0	RDW	13.4
				PLT	202
Normal WBC Pop				MPV	8.2
Normal RBC Pop					
Normal PLT Pop					

A-1 Histograms and printout generated by the Coulter STKR automated hematology analyzer. BA, basophil; EO, eosinophil; HCT, hematocrit; HGB, hemoglobin; LY, lymphocyte; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MO, monocyte; MPV, mean platelet volume; NE, neutrophil; PLT, platelet; RBC, red blood cell; RDW, red cell distribution width; WBC, white blood cell.



A-2 Run Screen generated by the *CELL-DYN® 3700* automated hematology analyzer. BASO, basophil; EOS, eosinophil; HCT, hematocrit; HGB, hemoglobin; LYM, lymphocyte; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MONO, monocyte; MPV, mean platelet volume; NEU, neutrophil; PLT, platelet; RBC, red blood cell; RDW, red cell distribution width; WBC, white blood cell.



A-3 Run Screen generated by the SYSMEX XE 2100 automated hematology analyzer.

GLOSSARY

Blood Smear

Is a thin layer of blood smeared on a microscope slide and then stained in such a way to allow the various blood cells to be examined microscopically.

Bone Marrow

Is the flexible tissue found in the interior of bones. In humans, marrow in large bones produces new blood cells.

FPGA

A field-programmable gate array (FPGA) is an integrated circuit designed to be configured by the customer or designer after manufacturing—hence "field-programmable". The FPGA configuration is generally specified using a hardware description language (HDL), similar to that used for an application-specific integrated circuit (ASIC) (circuit diagrams were previously used to specify the configuration, as they were for ASICs, but this is increasingly rare). FPGAs can be used to implement any logical function that an ASIC could perform.

Peripheral Blood

Peripheral blood is the flowing, circulating blood of the body. It is composed of erythrocytes, leukocytes and thrombocytes. These blood cells are suspended in blood plasma, through which the blood cells are circulated through the body. Peripheral blood is different from the blood whose circulation is enclosed within the liver, spleen, bone marrow and the lymphatic system.

RBC

Red blood cells (also referred to as erythrocytes) are the most common type of blood cell and the vertebrate organism's principal means of delivering oxygen (O₂) to the body tissues via the blood flow through the circulatory system.

VHDL

VHDL (VHSIC hardware description language) is a hardware description language used in electronic design automation to describe digital and mixed-signal systems such as field-programmable gate arrays and integrated circuits.

WBC

White blood cells (WBCs), or leukocytes (also spelled "leucocytes"), are cells of the immune system involved in defending the body against both infectious disease and foreign materials. Five different and diverse types of leukocytes exist, but they are all produced and derived from a multipotent cell in the bone marrow known as a hematopoietic stem cell. Leukocytes are found throughout the body, including the blood and lymphatic system.

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