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A New Electronic Instrumentation Approach for the Acquisition of Microscopic Blood Smear Images for the Automatic Diagnosis of Anemia

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Abstract

Anemia is a blood abnormality that affects the quantity and quality of red blood cells in the human body. This sometimes banal sign spares no continent and no social stratum. This anomaly is generally appreciated through biological analyzes of patients' blood. These analyzes, which boil down to the knowledge of hemato-metric constants, cannot by themselves allow the characterization of certain forms of anemia in the sense that most anemia are related to the morphology and color of red blood cells. Our work in this paper is to perform blood smears on patients and perform a morphological and colorimetric analysis of red blood cells on these smears. This approach allowed us to highlight on each erythrocyte morphological and colorimetric descriptors to accurately identify the types of anemia by image processing methods. This identification is performed in an automated environment to allow pathologists to respond quickly to anemia-related emergencies and also improve the treatment to be conducted. This automation required the implementation of a new approach to electronic instrumentation and the acquisition of microscopic blood smear images for the automatic and rapid diagnosis of anemia.

Keywords

Anemia, Instrumentation, Acquisition, Automated, Blood Smears, Red Blood Cells, Morphological Descriptors, Colorimetric Descriptors, Image Processing, Diagnosis

1. Introduction

With 1.62 billion people diagnosed worldwide, according to the database of the

World Health Organization (WHO), anemia is a major public health problem [1]. It touches every continent without exception. According to the report of the World Health Organization, globally the prevalence is 24.8% of the world population with a predominance of preschool children (47.4%) and a regression is observed in men (12.7%) [1]. But a high prevalence is observed in South Asia, Central Africa and West Africa. In Africa, for example, this prevalence is 62.3% among children aged 0 to 5 years [2]. Anemia threatens human existence because it affects about half a billion women of childbearing age in the world: In 2011, 29% (496 million) of non-pregnant women and 38% (32.4 million) of pregnant women aged 15 to 49 years were anemic [3]. Anemia spares no social layer in the world. This condition, which affects the number and/or quality of red blood cells (lowering of hemoglobin (Hb) levels in human blood) comes in many forms with a variety of causes.

Their characterization becomes paramount in that it can influence decision-making via diagnosis.

Automata already exist to perform the blood count which is an automated examination to assess certain types of anemia. Indeed, they make it possible to obtain information on the number and size of red blood cells [4]. But these two parameters do not provide enough information for a true characterization of anemia. Anemia can be categorized through the morphology and color of red blood cells (erythrocytes) observed on microscopic images obtained from blood smears performed on patients. Microscopic observation of blood smear images shows that there are different types of abnormalities related to the morphology and color of red blood cells [5]. Indeed, the combination of these two parameters will allow the detection of the type of anemia to clearly guide pathologists. Because depending on the shape or the color of the red blood cells we can affirm that it is about such a type of anemia and not of such another as the different images show it below (of **Figure 7** in **Figure 10**) in the result section.

In addition, medical personnel sometimes use manual methods to characterize anemia. This detection technique is rudimentary, difficult and very subjective (observation of the coloring of palms, conjunctivae and nails ...). For effective management of patients and to overcome the shortcomings of the various methods mentioned above, we propose a new instrumentation approach for the automatic and rapid diagnosis of anemia.

The work we are conducting reveals morphological and colorimetric descriptors that are discriminant extracted on each form of red blood cells. In this perspective, work has been done in the literature. Indeed, according to Chantal Fossat *et al.* in 2006 [6] the automated evaluators of schizocytes (fragments of red blood cells) present difficulties (limits) concerning the formal identification of these. The approach discussed in this paper may rule out some diagnostic assumptions or may highlight morphological abnormalities of erythrocytes for better diagnostic accuracy [7]. The detection of schizocytes (fragment of red blood cells) fortuitously on the blood smear is a key indicator for the diagnosis of thrombotic microangiopathic anemia, which represents a proven medical

emergency [8]. But the schizocyte search method still remains manual. In 2017, Hany A. Elsalamony, for the identification of sickle cell red blood cells, proposed a method based on the signature of their geometric form [9] [10]. Frejilichowski, 2011 developed a method for identifying abnormal red blood cells using a polar-Fourier grayscale descriptor on May-Grumwald-Giemsa stained blood smear images [11]. The compactness melting and the moment invariant of HU is the method used by R. Tomaria *et al.* 2014 to identify erythrocytes [12]. Das *et al.* in 2012 for the detection of certain forms of red blood cells used only certain morphological descriptors. Multi-class logistic regression is chosen because of its classification accuracy of normal and abnormal erythrocytes [13].

The implementation of an automated system according to Das *et al.* in 2012 [13] requires an instrumentation capable of performing a treatment of microscopic images resulting from blood smears. Then, the automatic extraction and the calculation of the different erythrocyte discriminating parameters morphologically and colorimetrically are carried out. The next article will aim to set up a method of classification or automatic recognition of the types of anemia suffered by a patient.

2. Materials and Methods

2.1. Materials

The characterization of the morphological and colorimetric of the red blood cells requires the production of blood smears on the various samples taken from healthy patients or from anemic patients. This realization requires the equipment of **Figure 3** below. The tools used for the realization of this study are apart from **Figure 3**, a computer, a microscope, a camera and software allowing us to capture microscopic images. This work will produce data in the field and data collected following the analysis of the images obtained. the data in the field will be essentially composed of those obtained by the automated machines of the laboratories and the laboratory data are collected following the analysis of microscopic images of blood smears which are parameters discriminating each form or colorimetric aspect of the red blood cell as indicated the characteristics calculated and grouped in the tables below.

2.1.1. Selection of Samples

The laboratory data come from the samples of thirty (30) healthy people and one hundred and twenty (120) anemic people. We carried out five (05) blood smears per patient and, after the visual analysis of the practitioners we chose two (02) blood smears respecting the recommendations of the World Health Organization (WHO) [14]. Is performed randomly and comes from the health services of the city of Yamoussoukro including the pediatrics, medicine, surgery and gynecology services of the regional hospital center (CHR) and also of the Transfusion Center (CTB) of said city. In order to respect the principles of our study, we took patients whose anemic state was proven or not following the field data provided by laboratory automata.

2.1.2. Perform a Blood Smear

The blood smear consists of spreading a drop of blood evenly on an object slide, so as to obtain a single layer on half of the surface of the slide [14]. After fixing and coloring, the morphological and colorimetric study of the figured elements of the blood can be carried out. To stick to our main objective, only red blood cells will interest us in this study. The different blood smears will each carry an identifier which corresponds to the patient from whom the sample is taken.

2.1.3. How to Prepare a Smear

- Mark the slide.
- Apply a drop of venous blood 1 cm from the tip of the object's blade, placed on a hard, horizontal plane.
- Hold the first slide with one hand and tilt the second slide 45° just in front of the drop.
- Gradually put the slide in contact with the drop of blood.
- Let the blood flow along the stop of the second blade and before it reaches the edges, in a fast, uniform and continuous movement, pull the blood forward.
- Dry quickly while shaking (**Figure 1**) [15].

2.1.4. Staining of a Blood Smear

There are two staining strategies with Giemsa: the fast method (10% dye) and the slow method (3% dye). The first is used in busy clinics and laboratories where speed of diagnosis is an essential element in the management of patients. The slow method is used to stain more slides, as is the case in epidemiological surveys. Given the large influx in the laboratories and the expected result we preferred the staining method to 10%.

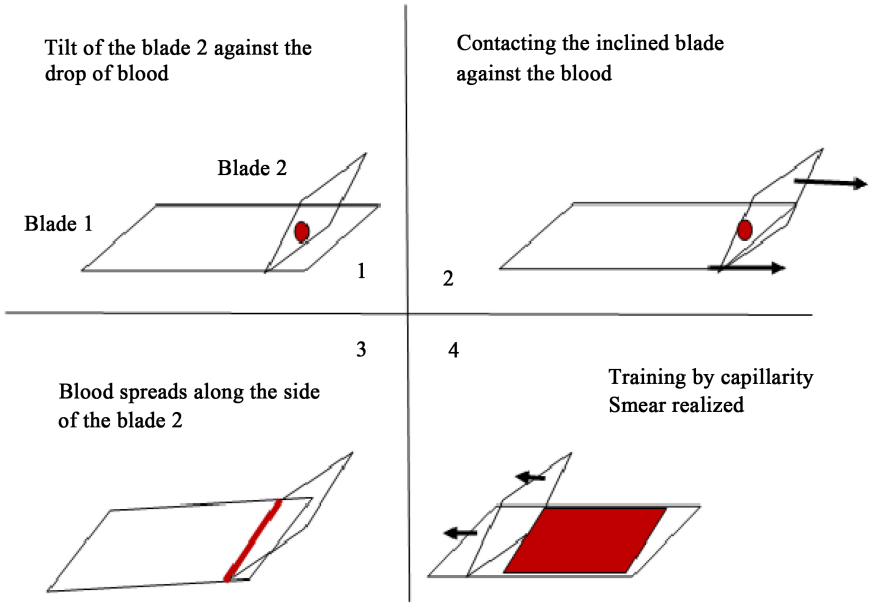


Figure 1. Demonstration of the blood smear technique

There are usually two techniques for staining blood smears (by overlay and bath). We chose the bath staining technique instead of the stain technique. This technique has the advantage of staining several blood smears at a time, which is what guided our choice.

The process of bath staining of blood smears consists in following in chronological order the following steps:

- Preparation of three jars, the first containing a pure solution of May-Grunewald. the second containing a buffer solution and finally the third jar a solution of Giemsa diluted 1/10.
- Dive into the first jar of blood smears for five minutes (5 min).
- Transfer of blood smears into the jar containing the buffer solution.
- Removal of the smears from the buffer solution and soaking for 15 minutes in the jar containing Giemsa diluted 1/10 with distilled water pH 7.2.
- Rinse thoroughly 2 - 3 times each slide under running water.
- Exposure of the blades to the open air for drying.
- Wait for complete drying before the acquisition (**Figure 2** and **Figure 3**).

2.1.5. Acquisition of Microscopic Images

The microscope and the Moticam 2.0 camera are mounted to make it a single entity. The block thus obtained is connected with a USB cable to the microcomputer on which the Moticam 3.0 software is installed, see **Figure 4** below. This set will promote the automatic acquisition of microscopic color images of the blood smear. These images will be scanned and stored on the microcomputer with the installed software.

Figure 2. Example of non-colored blood smear.

Figure 3. Colored smears for acquisition images.
https://pixers.fr/tableaux-sur-toile/frottis-sanguin-FO34994755.

Figure 4. Image acquisition device.

1) The Characteristics of the Equipment

Capturing microscopic images for the proper conduct of our work required the acquisition of the following equipment: a microscope, a camera and a laptop. The characteristics of the camera and the microscope are grouped respectively in Table 1 and Table 2 below.

1) The Characteristics of the Computer

The entire device runs on an hp laptop with the following features:

- Processor: Intel® Core™ i3-5005U processor at 2.00 GHz at 2.00 GHz.
- Operating system: Windows10 Professional.
- RAM: 4, 00 Go.
- System type: 64-bit operating system, ×64 processors.

2.2. Methods

The proposed method starts from the acquisition of microscopic color images of the selected blood smears. Then we treat and characterize the different morphological and colorimetric parameters of each form of red blood cell (**Figure 5**).

Morphological and Colorimetric Characterization

The morphological and colorimetric characterization of red blood cells requires the extraction of different discriminating descriptors for each form of red blood cells. Indeed, the recognition of red blood cell forms is based on measurable data extracted on red blood cells. These data or characteristics must be discriminating in order to achieve good identification [16]. In this section we will present for each red cell the appropriate descriptor.

In this approach we need to isolate the discriminating red blood cell by an image processing tool that has segmentation.

1) Segmentation

Segmentation is the partition of an image into several regions according to a well defined criterion and having pixels of the same characteristics. The main purpose of this treatment is to extract the information that must allow a precise identification of the object concerned [16]. In the literature several segmentation methods have been developed to identify blood cells [9] [13] [17] [18]. In 2015,

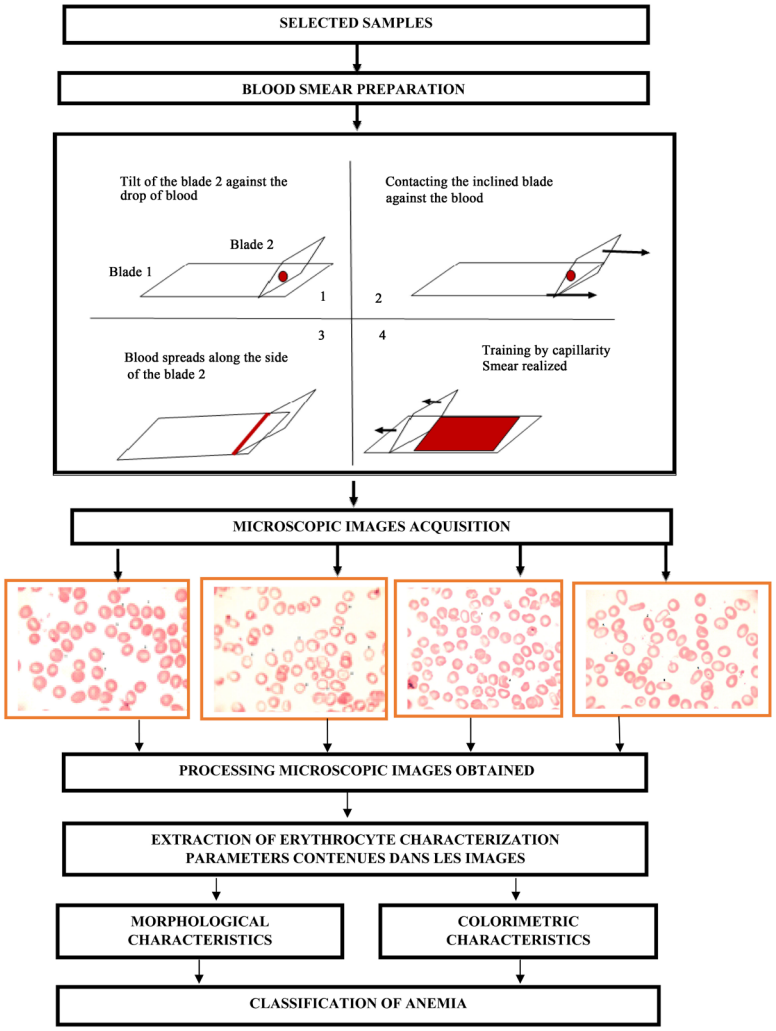


Figure 5. Flowchart of the proposed methodology.

Table 1. The characteristics of the camera.

Parameters values	Values
Moticam	2
Sensor type	CMOS
Optical	1/3"
Actives pixels	1600 × 1200
Pixel size	3.2 μm × 3.2 μm
Imaging Area	5.12 mm × 3.84 mm
Scan Mode	progressive
Shutter type	Rolling with Global reset
Operating temp	−10˚C to 60˚C non condensing
Max. SNR	43 dB
D/R	61 dB
Interface	USB2.0
Operating req.	Windows7 or higher, osx, Linux, 250 MB free HD space

Table 2. Characteristics of microscope.

Parameters	Values
Mechanism Tube length	160 mm
Viewing Head	Compensation Free Trinocular Head, inclined at 30, interpupillar distance 55 - 75 mm
Eyepiece	Viewfield line 18 mm
Nosepiece	Forward Quadruple Nosepiece
Object	Achromatique: 4×, 10×, 40×, 100×
Focus System	Coaxial coarse and Fine Focusing System, sensitivity and Graduation of Fine Focus: 0.002 mm. coarse & fine focus range: 23 mm
Condenser	Abel, NA = 1.25
Stage	Double layer mechanical stage, area: 140 × 140mm, movement range: 75 × 50 mm
Lamp-House	Halogenlamp 6 V 20 W

the study by P. Shivhare *et al.*, listed three main methods of segmentation [16]:

- Edge-based segmentation
- Segmentation based on the region
- Segmentation based on thresholding

a) Edge-based segmentation

This method is based on the abrupt change in pixel luminance that determines the contour. It makes it possible to mark a border or a real transition zone between the regions (the objects) and the background of the image.

b) Segmentation based on the region

The region in an image is the grouping of a number of pixels having similar values. This method then makes it possible to group a number of homogeneous pixels or having common attributes [19].

c) Segmentation based on thresholding

Thresholding segments an image into two classes. The intensity of each pixel is compared to the defined threshold and that pixel by pixel. When this intensity is below the threshold, the pixel takes the value 0 and the value 1 otherwise. This thresholding operation applied to the entire image leads to binarization of the image [20].

2) Morphological descriptors

Healthy red blood cells have a uniformly rounded and undeformed shape whereas those that are anemic have various forms that are often specific to the type of anemia. This morphological deformation can be characterized by shape descriptors:

a) Area of the red blood cells

The surface of the red cell or area is the set of pixels covering the segmented image.

$$\text{area} = \sum_x \sum_y f(x, y) \tag{1}$$

$f(x, y)$ is the pixel whose position is represented by the pair of x and y coordinates in the binarized image. It is 1 when the pixel is in the segmented region and 0 otherwise.

b) Perimeter of the red blood cells (P)

The perimeter is the sum of the pixels on the edge of the segmented image. To calculate it we will use the eight-connexity method (Table 3).

We go through the whole contour. When a pixel has a connectivity lower than 8 this pixel belongs to the outline.

$$P = \sum_x \sum_y f(x, y) \tag{2}$$

c) Compactness (C)

The compactness of the region is the ratio between the area of this region and the perimeter. It measures the regularity of the surface of the region

$$C = \frac{4 \times \pi \times \text{area}}{p^2} \tag{3}$$

d) Eccentricity (e)

Eccentricity describes the degree of elongation of a red blood cell.

$$e = \left(1 - \frac{b^2}{a^2}\right)^{1/2} \tag{4}$$

The variable b represents the minor axis and the variable a represents the major axis. The value of eccentricity varies between 0 and 1 for red blood cell forms. When $e = 0$ the object is practically a circle, when $e < 1$ the object is lengthened.

e) Convex set

Let two distinct pixels x and y belong to the same red cell $(\mathcal{E})$. The number of pixels separating them describes a segment $[x, y]$. If the entire segment belongs to the cell whatever x and y then the set is convex. This method allows us to clearly characterize certain erythrocytes.

$$\left\{(\mathcal{E}) \text{ convex if } \forall x, y \in (\mathcal{E}), tx + (1-t)y, t \in [0,1]\right\} \tag{5}$$

The segment [x, y] is defined as follows:

$$\left\{(x, y) / tx + (1-t)y, t \in [0,1]\right\} \tag{6}$$

t is the variable describes the segment $[x, y]$

3) Color Descriptors

a) Average pixel intensity

An image is a set of pixels, each of which has a value that defines its intensity. So the average intensity of this region can be known. We have therefore from the

Table 3. The eight-connexity neighbours of the pixel (x, y).

$(x-1, y-1)$	$(x, y-1)$	$(x+1, y-1)$
$(x-1, y)$	**(x, y)**	$(x+1, y)$
$(x-1, y+1)$	$(x, y+1)$	$(x+1, y+1)$

algorithm below calculated the average intensity of the pixels for each region. The algorithm is designed under MATLAB 2016a. Its principle is defined hereafter:

b = view image (filename);

im = read image (filename);

im2 = converting colors to grayscale (im);

h = selection region;

bw = conversion to binary image (h, b);

view image (bw);

Imoy = Mean color of object in white.

b) Entropy

Entropy is a statistical measure to obtain information. It becomes an important parameter to characterize an image.

$$H(x) = \sum_{i=1}^{n} P_i \cdot \log_2(P_i) \tag{7}$$

c) Standard deviation (σ)

This statistical tool is the gap between the intensity of each pixel to the average of the pixel intensity.

$$\sigma = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(x_i - \bar{x})^2} \tag{8}$$

d) Percentage of colored area in red (PCol)

The colored part of the red blood cell contains hemoglobin hence the red color of the cells.

$$\text{PCol} = \left((\text{areag} - \text{areab})/\text{areag}\right) * 100 \tag{9}$$

Areag: represents the number of pixels of the total area of the red blood cell,

Areab: the number of pixels of the central white part of the erythrocyte,

PCol: represents the percentage of ocqupation of the colored area of the red blood cell.

e) Area of the white zone of the red blood cells

$$\text{areablc} = \text{areag} - \text{areacol} \tag{10}$$

$$\text{pblc} = (\text{areablc}/\text{areag}) \times 100 \tag{11}$$

Areablc: is the set of pixels in the central area of the red cell,

Areacol: the set of pixels in the colored area.

3. Results and Discussions

Examination of the blood smear makes it possible to carry out a morphological and colorimetric study of the various figured elements of the blood. For our work, only red blood cells interest us. On a normal smear, red blood cells have rounded shapes, without nuclei and the same color. Any modification of these parameters reflects a pathological condition as shown in **Figures 8-10** below. Anemia is a consequence of these changes.

In this section, we will present the results obtained during the acquisition and identification phase of red blood cells.

3.1. Some Acquisitions of Blood Smear Images from Different Patients

For each blood smear we made images of several optical fields of different patients. In order to obtain quality and exploitable images, we played on certain parameters of the camera. These are the following parameters (Figure 6):

- Color (Gain and brightness of RGB colors).
- Brightness.
- Resolution, exposure, contrast, gain.
- White balance, gamma.

We present in this section some images. Figure 7 below shows a normal smear image characterized by circular, coreless red blood cells with a slightly white area in the center. These different cells have approximately the same color and the same morphology. Such a smear shows the absence of anemia.

Anemia with hypochromic erythrocytes is anemia that is caused by iron deficiency in the body. It is also called iron deficiency anemia. This type of anemia is common in medicine. It is characterized by a pallor of red blood cells with the white central area more developed and it is found that the hemoglobin (red part) is deposited at the periphery of the red blood cells forming a ring as shown in Figure 8 below. It can also be observed in chronic hemorrhages (gastric ulcers, gastric cancer, hemorrhoids ...).

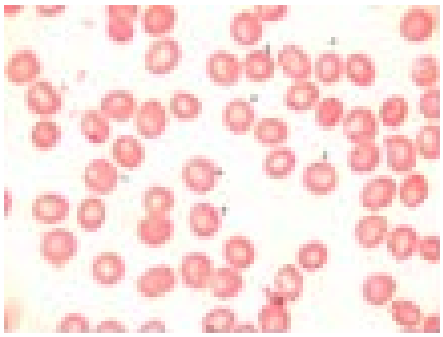


Figure 6. Smear image showing rounded red cells with the same red color.

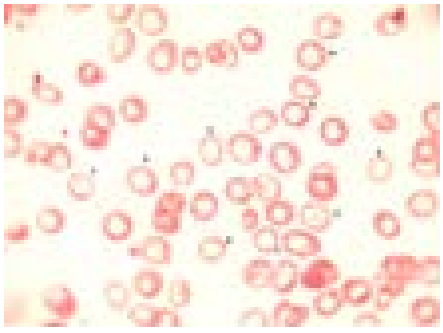
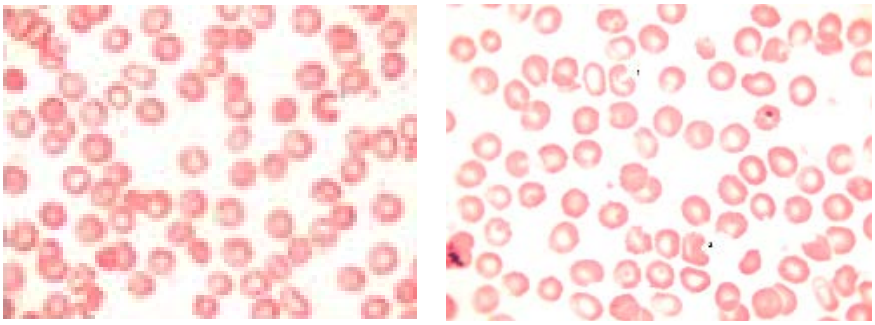


Figure 7. Image showing pale red blood cells, low in hemoglobin (hypochromia).



(a) (b)

Figure 8. Numbers (1) and (2) of Figures (a) and (b) show sickle cells.

Figure 9 below shows deformed red blood cells in the shape of a sickle or banana. They are responsible for sickle cell disease, which is a disease of the hemoglobin (A and S) genes. The gene A is said to be normal, whereas the S gene is responsible for the deformation of the red blood cell skeleton in the shape of a sickle or banana. The detection of such a morphological abnormality is possible on the blood smear.

Figure 10 shows the image of a blood smear on which red blood cells numbered 1 - 6 have elongated shapes with rounded ends: they are elliptocytes. This form of red blood cells may be present in the healthy subject (less than 1%) but in the case of hereditary elliptocytosis the number of elliptocytes may vary from 10% to 90% of the red blood cells [21]. The optical images were obtained with the objective 100 times (100×), which is equivalent to a magnification (G) of 1000 (eyepiece: 10× and objective 100×, *i.e.* G = 10 × 100), allowing a morphological and colorimetric study of the Red blood cells.

3.2. Images of Some Segmentation and Measurement of Morphological and Colorimetric Parameters on Red Blood Cells

The characterization of a red blood cell requires both measured morphological and colorimetric data. The success of this action leads to the use of an image processing tool: segmentation. The advantage of this tool is to isolate the cell in order to extract information that can identify it accurately. Segmentation methods are numerous [16] as defined in Section 2.4.1. But to extract information from each of the red blood cells we propose a method of segmentation semi-supervised by the selection of the contours of each cell. We segment the isolated red blood cell three times, then record the different measurements and average them as shown in **Tables 4-11**. This method will result in isolation of the cell as shown in **Figure 10(a)** and **Figure 10(b)**. We find that the images in **Figure 10** highlight the cell in white on a black background. All the information to extract is in the white part that represents the segmented red blood cell. This information is obtained through algorithms that we developed and implemented under MATLAB 2016a. The results obtained on each erythrocyte or red blood cell are recorded in a table, some of which are presented in **Tables 4-11** below.

Table 4. Morphological parameters of healthy red blood cell.

cap269	area	perimeter	compactness	eccentricity	pbinary
H1	5559	274	0.93048	0.3400	0
	5557	270	0.95791	0.2910	0
	5466	273	0.92162	0.3212	0
MH1	5527.3333	272.3333	0.93667	0.3174	0
H2	5416	273	0.9132	0.2803	0
	5400	267	0.9519	0.3287	0
	5598	272	0.9508	0.1793	0
MH2	5471.3333	270.6667	0.9386	0.2628	0
H3	5727	278	0.9312	0.3823	0
	5800	279	0.9363	0.3162	0
	5717	274	0.9569	0.2847	0
MH3	5748	277	0.9415	0.3278	0

Table 5. Colorimetric parameters of healthy red blood cells.

cap269	intMoy	std	colMR	ColMG	ColMB	%colored	%white
	202	5.1568	255	224	220	94.2616	5.7384
H1	202	5.1547	255	224	220	94.3134	5.6865
	202	5.1153	255	224	220	94.1090	5.8910
MH1	202	5.1423	255	224	220	94.2280	5.7720
	204	5.7267	255	224	220	83.4564	16.5436
H2	204	5.7797	255	224	220	84.0741	15.9259
	204	5.8043	255	224	220	84.8875	15.1125
MH2	204	5.7702	255	224	220	84.1393	15.8606
	204	5.4969	255	224	220	90.3964	9.6036
H3	204	5.5139	255	224	220	90.1724	9.8276
	204	5.4615	255	224	220	89.9948	10.0052
MH3	204	5.4908	255	224	220	90.1878	9.8121

Table 6. Annulocyte morphological parameters.

Cap311	area	perimeter	compactness	eccentricity	pbinary
	4171	241	0.9024	0.4940	0
H1	4123	234	0.9462	0.5723	0
	4036	235	0.9184	0.3815	0
MH1	4110	236.6667	0.9223	0.4826	0
	3403	215	0.9251	0.4013	0
H2	3558	220	0.9238	0.4806	0
	3428	213	0.9495	0.4523	0
MH2	3463	216	0.9328	0.4447	0
	3975	229	0.9525	0.3500	0
H3	4294	240	0.9368	0.2797	0
	4097	234	0.9402	0.3008	0
MH3	4122	234.3333	0.9432	0.3102	0

Table 7. Annulocyte colorimetric parameters.

Cap311	intMoy	std	colMR	ColMG	ColMB	%colored	%white
	228	18.2366	254	223	212	60.1295	39.8705
H1	228	18.4479	254	223	212	62.6728	37.3272
	228	18.4187	254	223	212	59.6878	40.3122
MH1	228	18.3678	254	223	212	60.8300	39.1700
	219	25.7382	254	223	212	62.8857	37.1143
H2	219	25.5280	254	223	212	62.4227	37.5773
	219	25.7352	254	223	212	62.3396	37.6604
MH2	219	25.6671	254	223	212	62.5493	37.4507
	219	24.9620	254	223	212	59.4214	40.5786
H3	219	24.4978	254	223	212	62.8319	37.1681
	219	24.7150	254	223	212	61.5572	38.4428
MH3	219	24.7249	254	223	212	61.2702	38.7298

Table 8. Morphological parameters sickle cell.

Cap227	area	perimeter	compactness	eccentricity	Pbinary
	2855	278	0.4642	0.8672	1
H1	2981	276	0.4918	0.8813	1
	2971	275	0.4937	0.8553	1
MH1	2935.6667	276.3333	0.4832	0.8680	1
	2208	207	0.6475	0.7560	1
H2	2098	201	0.6526	0.8140	1
	2183	207	0.6402	0.8329	1
MH2	2163	205	0.6468	0.8010	1
	3295	271	0.5638	0.8413	1
H3	3358	275	0.5580	0.8372	1
	3309	268	0.5790	0.8346	1
MH3	3320.6667	271.333	0.5669	0.8377	1

Table 9. Sickle cell colorimetric parameters.

Cap227	intMoy	std	colMR	ColMG	ColMB	%coloré	%blanc
	175	16.8154	255	222	215	100	ABS
H1	175	18.3393	255	222	215	100	ABS
	175	18.1241	255	222	215	100	ABS
MH1	175	17.7596	255	222	215	100	ABS
	184	20.8689	253	214	204	100	ABS
H2	184	20.0974	253	214	204	100	ABS
	184	20.5583	253	214	204	100	ABS
MH2	184	20.5082	253	214	204	100	ABS
	197	13.2418	255	213	206	100	ABS
H3	197	14.3507	255	213	206	100	ABS
	197	13.8554	255	213	206	100	ABS
MH3	197	13.8159	255	213	206	100	ABS

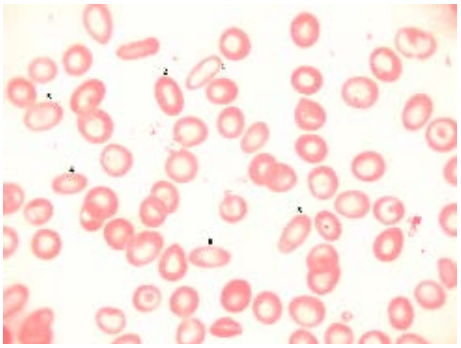


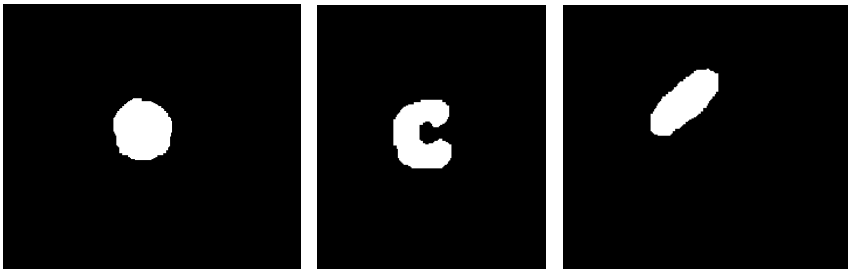
Figure 9. Smears with numbered red blood cells elliptical shapes called elliptocytes.

Table 10. Morphological parameters elliptocytes.

Cap229	area	perimeter	compactness	eccentricity	Pbinary
	4013	251	0.8004	0.8727	0
H1	3923	245	0.8213	0.8738	0
	4029	251	0.8036	0.8781	0
MH1	3988.33	249	0.8085	0.8749	0
	4500	252	0.8905	0.7476	0
H2	4801	267	0.8463	0.7860	0
	4801	267	0.8463	0.7860	0
MH2	4700.667	262	0.8610	0.7732	0
	4272	256	0.8191	0.8513	0
H3	4210	259	0.7887	0.8739	0
	4211	253	0.8267	0.8522	0
MH3	4231	256	0.8115	0.8591	0

Table 11. Elliptocyte colorimetric parameters.

Cap229	intMoy	std	colMR	ColMG	ColMB	%colored	%white
	232	17.1237	255	233	228	68.9758	31.0242
H1	232	17.1677	255	233	228	67.0150	32.985
	232	17.0458	255	233	228	68.3544	31.645
MH1	232	17.1122	255	233	228	68.115	31.8849
	208	19.5177	255	233	228	82.2222	17.7778
H2	209	20.9228	255	233	228	82.9410	17.0589
	209	20.9228	255	233	228	83.2118	16.7882
MH2	208	20.4545	255	233	228	82.7917	17.2083
	218	15.5455	255	233	228	80.2434	19.7565
H3	218	15.4646	255	233	228	80.9263	19.0736
	218	15.0689	255	233	228	81.0021	18.9978
MH3	218	15.3597	255	233	228	80.7239	19.2760



(a) (b) (c)

Figure 10. (a) Segmentation of a circular red blood cell; (b) Segmentation of a sickle red blood cell; (c) Segmentation of an elliptical red blood cell.

The tables are divided into two groups for each segmented red blood cell. In a first table are morphological descriptors and in a second color descriptors. In each table the horizontal side contains the following descriptors: area: represents the number of pixels lying on the whole surface of the segmented cell (white part in **Figure 10(a)** and **Figure 10(c)** above), the perimeter which is the sum of the pixels is lying on the contour of the segmented cell, the compactness, eccentricity and convexity of the cell (red blood cell). In the second table we find the average intensity (**intMoy**) of the pixels, the standard deviation, the average color (ColMR, ColMG, colMB) in each RGB component and the percentages of the white (% white) of the center of the red blood cells and the colored part At the vertical level, capXXX indicates the identifier of each acquired image, H {1, 2, 3, ..., n} represents the set of segmented red blood cells and MH {1, 2, 3, ..., n} indicates the average of the measurements made on each red blood cell. On healthy patients morphological and colorimetric measurements were made on the red cells of the images acquired. It can be seen that the red blood cells are circular, which is justified by their compactness values varying between 0.9 and 1. Healthy red blood cells are practically the same size in view of their perimeters which is around 272 as indicated in **Table 4** above. The color of the healthy red blood cells is practically red this is noticed by the average value calculated in each channel of the RGB space (R = 250, G = 224 and B = 220). This observation is confirmed by the low standard deviation (STD) whose value is around 5 which reflects the homogeneity of the red blood cell. This density of the red color is justified by its high proportion which varies from 84% to 94% this observation is made through the data in **Table 5** above.

Table 6 above contains the morphological parameters of annulocytes. We find that the parameters are virtually identical to those in **Table 4** above see compactness, eccentricity, and the Pbinary convexity variable. So, in order to precisely identify the annulocytes we associate with the morphological parameters the colorimetric parameters of **Table 7** above because without the color aspect it could be confusing. In terms of colorimetric parameters, we find a low density of the red color which is around 61% which is lower than the values of the healthy red blood cells of **Table 5** (84% to 94%). The white zone in an annulocyte (39% of the area of the red blood cell) is almost 8 times the white area of a healthy red

blood cell (5% of the area of the red blood cell). These values were obtained by semi-automatic contour segmentation to calculate the area of the white area inside the cell (Section 2.4.2 d-c).

In sickle cell patients red blood cells are sickle-shaped cells or banana cells. Like all acquired images we have made morphological measurements grouped in Table 8 above. At the analysis we find that the cells do not circular in view of the value of their compactness which vary between 0.4 and 0.6. They are also elongated and this is justified by their values of eccentricity ranging from 0.7 to 0.8 but this descriptor is not efficient enough because in our work we have also found that the elongated cells called elliptocytes have the same eccentricities as the sickle cells observation made in Table 10 above. For a more precise identification we used the convex set marked by the value of the variable ***PBINARY***. This value is 1 if the object is non-convex and 0 if the object is convex. When we associate compactness with the study of the convex set of sickle cell cells, our system makes it possible to clearly identify the sickle cell. The coloring of the sickle cells is substantially red, which is **H1**: first red blood cell, ***MH*1**: mean red blood cell measurements H1, ***INTMOY*** average pixel intensity, ***colMR***, ***ColMG***, ***ColMB***, average staining of ***RGB*** components, **STD**: standard deviation**,% COLOR and% WHITE**, percentage of the colored part and the white part of the red blood cells.

Elliptocytes are elongated red blood cells with rounded ends. Figure 9 above that is justified by their compactness values varying between 0.7 and 0.9. Their elongated shape is also justified by their eccentricity values which vary between 0.7 and 0.9 (eccentricity less than 1). The identification of this cell form will be done with the combination of its compactness and eccentricity which are two descriptors discriminating for this form this analysis is made through the data measured in Table 10 above. The colorimetric parameters related to this form in Table 11 above shows that some elliptocytes have clear central zones that are more developed than others. Erythrocyte1 (***H*1**) has 31% white and 69% colored and erythrocyte2 (***H*2**) has 17% white and 83% white. The instability of the coloration shows that the color is not a discriminating factor for this form. Then only the morphological parameters will lead us to a good identification of the elliptic shapes.

H1: first red blood cell, MH1: mean red blood cell measurements **H1**, **intMoy:** average pixel intensity, ***colMR, ColMG, ColMB***, average staining of RGB components, **STD:** standard deviation**, % COLOR** and **% WHITE:** percentage of the colored part and the white part of the red blood cells.

4. Conclusion

In this paper, we propose a new instrumentation approach to automatically characterize different anemia. The work done in this document consisted mainly of elaborating patient eligibility criteria for our study. Then we collected our samples and prepared the blood smears. The acquisition phase took place in an en-

vironment described in this paper. For a better identification of each of the forms of red blood cells specific to anemia types, we propose efficient and specific descriptors for both the geometric shapes and the color appearance of red blood cells. Thus, for a formal identification of shapes, we associated the geometric descriptors (area, perimeter, compactness, eccentricity, the convex set) with the colorimetric descriptors (mean pixel intensity, standard deviation, average color for each RGB component, percentage color and white). This combination of parameters allows good identification. The extraction of these morphological and colorimetric characteristics of normal and abnormal red blood cells was possible thanks to our semi-supervised contour selection segmentation method and to the algorithms we developed and implemented under Matlab 2016a. Our future work will concern the further identification of certain forms of red blood cells and will end with the classification of anemias based on the morphology and color of red blood cells.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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