Asociación Argentina
de Mecánica Computacional

Mecánica Computacional Vol XXXVI, págs. 441-447 (artículo completo)
José G. Etse, Bibiana M. Luccioni, Martín A. Pucheta, Mario A. Storti (Eds.)
San Miguel de Tucumán, 6-9 Noviembre 2018

DEVELOPMENT OF A SIMPLE METHODOLOGY BASED ON FRACTAL MATHEMATICS FOR SELECTIVE DIAGNOSIS OF RED BLOOD CELLS DISORDERS

Alcides J. Leguto[a], Juan P. Rebechi[a], Manuel A. Mancilla Canales[a,b], Patricia Ponce de León[a], Santiago A. Bortolato[a,c], Susana Pérez[a] and Ana M. Korol[a,b]

[a]*Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 570 Rosario, Argentina.*

[b]*Instituto de Física Rosario (IFIR), CONICET, Ocampo y Esmeralda, Rosario, Argentina.*

[c]*Instituto de Química Rosario (IQUIR), CONICET, Suipacha 570, Rosario, Argentina.*

Keywords: Box-counting dimension, wavelet, red blood cells, leukemia.

Abstract. Automatic characterization of different populations of red blood cells (RBCs) is a useful tool in Hematology and Clinical Diagnosis. In this work we focus on different pathologies that affect hemorheological properties of humans being blood. Our main aim is to develop an analytical methodology to aid in the diagnosis of those pathologies that undergo specifically by altering RBCs membrane dynamic properties. The alterations on the RBC membranes were studied with box-counting dimension (BCD). BCDs were estimated by a standardized analysis of denoised images of RBC suspensions obtained with an optical microscope and a non-professional digital camera. The systematical denoising was carried out by application of Wavelet Transform on the images. BCD is a fractal quantifier that has been proven to depend on the levels of RBC aggregation. Wavelet Transform denoising technique implies the decomposition of the image signals in a set of wavelets and the selection of the most significant ones through which the image can be reconstructed. In this work we compared the BCD estimated on RBC suspensions from healthy individuals and from those affected by parasitosis (trichinosis and ascariasis), leukemia (chronic and acute) and iron-deficiency anemia. In comparison to control samples, those corresponding to patients with parasitosis and acute leukemia showed significative differences ($p < 0.01$) in the variances of their BCD values, while the samples corresponding to anemia and chronic leukemia showed significatively higher ($p < 0.01$) BCD mean values.

1 INTRODUCTION

Blood is a type of connective tissue with a number of biological functions, most of them carried out by the so called “blood cells”. White blood cells (WBCs) or leucocytes are related to functions of the immune system. Red blood cells (RBCs) or erythrocytes are the most abundant cells in the blood (~ 99%) and their primary function is to transport respiratory gases between the lungs and other body tissues. RBCs are also major determinants for the rheological behaviour of blood because of their mechanical properties and abundance (Chien, 1987).

Several conditions affecting erythrocytes are known to alter these rheological properties, and it has been proposed that those alterations are responsible for different pathologies. In this work we will focus in parasitosis and anemia, which directly alter the properties of RBCs, and in leukemia, disease that is suggested to affect erythrocytes in an indirect manner.

Trichinella spiralis (Ts) is a pathogen parasite involved in the development of trichinosis. This disease is transmitted by food and is endemic in Argentina. Studying the alterations in erythrocyte aggregation by image analysis could be a feasible alternative that could provide more information to the knowledge of the parasite interaction with RBCs. *Ascaris lumbricoides* parasites produce ascariasis, one of the most widespread parasitic diseases in the world. Its most characteristic effects are perforation of the intestinal wall and pulmonary abscesses.

Leukemia refers to a group of bone marrow cancers that result in a high number of abnormal white blood cells, with a concomitant diminution in the quantity of erythrocytes. These types of cancer can be either acute or chronic. For the acute form, immature blood cells are rapidly produced and accumulated, making the bone marrow unable to produce healthy blood cells. In the case of chronic leukemia, there is an excessive production of relatively mature, but still abnormal, white blood cells which can induce symptoms several months or years after the early stages of the disease.

Anemia is a disease caused by a decrease in the number of red blood cells or the amount of hemoglobin in the blood. Specifically, iron-deficiency anemia is characterized by low blood concentrations of iron, which is a necessary cofactor of hemoglobin.

The proposed analysis is based on fractal patterns measured at different portions of digital images of RBCs. In general, we could define a fractal as a geometric figure which exhibits similar patterns at different scales i.e., a self-similar pattern. A commonly used parameter to quantify the degree of self-similarity is Fractal Dimension. Fractal dimensions cannot be calculated accurately but can be estimated. A widespread strategy to estimate it is box counting, a technique that can be applied to any distribution points, curves, surfaces, etc. to obtain the Box-Counting Dimension (BCD). Box Counting has been applied in distinct areas of knowledge, as it represents an easy and trustworthy approach for studying pictures or photos. In our case, we have previously applied this technique for discrimination of different groups of RBCs according to their nature, differentiating healthy from ill RBCs (Lupo, 2016).

Wavelets are wave-like oscillation with non-zero amplitude only during a short interval. Wavelet Transform (WT) is a time-frequency transform that decomposes a *n*-dimensional signal into a representation that shows signal details and trends as a function of time. In comparison with Fourier transform, wavelets are localized in both time and frequency, allowing the application of WT to new real physical situations in which a signal contains discontinuities or sharp spikes. Some applications of WT include data and image compression, partial differential equation solving, pattern recognition and noise/trend reduction.

In previous works, we have intended to demonstrate how larval parasites interact with RBCs to generate erythrocyte aggregation, and how this interaction can be measured using a mathematical approach (Lupo, 2016; Mancilla-Canales, 2017). Quantitative description of erythrocyte aggregation phenomena had been previously described, but with no conclusive results owing to the complex features of biological systems (Rapa, 2005; Talu, 2016). In the present work, we use WT in order to reduce noise from images prior to applying the box counting method, accounting for modifications in BCD values without sensitively affecting the images appearance. This approach allowed us to discriminate the effects caused by parasitosis, iron-deficiency anemia and different types of leukemia over the RBCs aggregation. The proposed method only requires microscopic pictures of RBCs suspensions, which can be obtained by a non-professional camera, thus representing a simple low-cost strategy for clinical diagnosis in low-resources laboratories.

2 MATERIALS AND METHODS

2.1 Red blood cells (RBCs)

Any person involved in this work have signed a medical informed consent form for participating in this research work. This project was approved by the ethics committee of the Rosario National University (Resolution number: 033/2015).

Fresh group O blood samples were obtained from donors and conserved in a container holding EDTA as anticoagulant, stored at 4°C and analysed within 24 hours. After removing autologous plasma, RBCs were washed three times with phosphate-buffered saline (PBS) (pH = 7.4, 295 ± 8 mOsmol / kg).

For the study of leukemia and anemia, the samples were obtained from patients affected by those diseases. For the study of parasitosis, blood samples from healthy donors were incubated with parasitic larvae. Adult parasitic extracts of *Ascaris lumbricoides* were used at concentrations of 5100 ± 200 larvae/mL. They were obtained by cuticle surgical removal and refrigerated mechanical rupture, following the protocol described in Ponce-de-León *et al*, 2013. Infective larvae of *Trichinella spiralis* obtained from muscle of infected mice were released by artificial digestion using pepsin and hydrochloric acid. The larvae were concentrated by centrifugation and counted by duplicate. Five larval concentrates were prepared with an amount of muscle larvae of 4300 ± 200 larvae/mL. The erythrocyte pellet was incubated with equal volume of the larval concentrate for 60 min at 37°C. The RBCs used as control were incubated only with PBS. After the incubation period, the treated and control RBCs were washed with PBS.

2.2 Digital Image Analysis

RBCs were suspended in 0.13% autologous plasma and maintained at rest for 5 minutes to allow aggregation. RBCs samples were examined in a concave optical inverted microscope slide (Union Optical, Japan). Images for each sample were obtained by duplicate (objective: 40X, Canon Powershot A640 digital camera). Images were then processed with the software Fractalyse, in order to calculate the Fractal dimension (D), according to an iterative principle based on the Box - Counting method.

3 DATA ANALYSIS

3.1. Box – Counting Dimension

Box counting dimension is related to how information accommodates and is distributed in an image (Grassberger, 1990). The fractal must be placed on an evenly spaced grid, and then count how many boxes are required to cover the set. The Box - Counting dimension depends on how this number changes as the grid gets finer by applying a Box - Counting algorithm.

Suppose that $N(L)$ represents the number of boxes of side L required to cover the set X ($X \in R^2$). Then the box counting dimension is defined as:

$$Dim(X) = \lim_{L \to 0} \frac{\log N(L)}{\log(\frac{1}{L})}$$

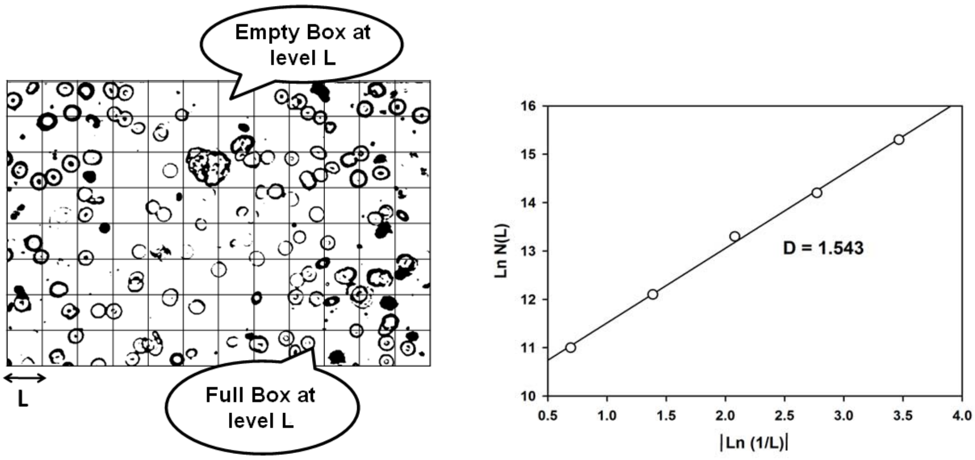

Figure 1. Representative example of application of Box-Counting method. (Left) Black-and-white picture with a guide grid. (Right) Box-Counting plot.

Results are showed on a graph where abscissas represent the absolute values of the logarithms of reciprocal L, (Ln L^{-1}), and ordinates correspond to logarithm of N. Therefore, the slope of the resulting line is the Box-Counting Dimension. If the obtained relationship does not fit a power law, the object is not self-similar or fractal.

3.2. Wavelet Transform

The process to remove noise from an image is based on the decomposition of the observed signal in a set of wavelets, the wavelet family, and then in the selection of a threshold value in order to select the appropriate coefficients to reconstruct the signal. An additional factor to consider is the selection of the wavelet. In this study Biorthogonal Wavelets (order 3, level 1.1) were used since they showed better results in the reconstruction of the images from their Inverse Wavelet Transform without altering the key features of the processed images.

3 RESULTS AND DISCUSSION

The first column of Figure 2 shows pictures of RBCs suspensions corresponding to control blood samples and affected by parasitosis, anemia and leukemia. In the second column the automatic transformation to black and white is shown, while the third shows the wavelet filtered images. This filtration technique decrease the dispersion of estimated BCD values in a ~10% by eliminating the noise that can account for errors in Box-Counting method.

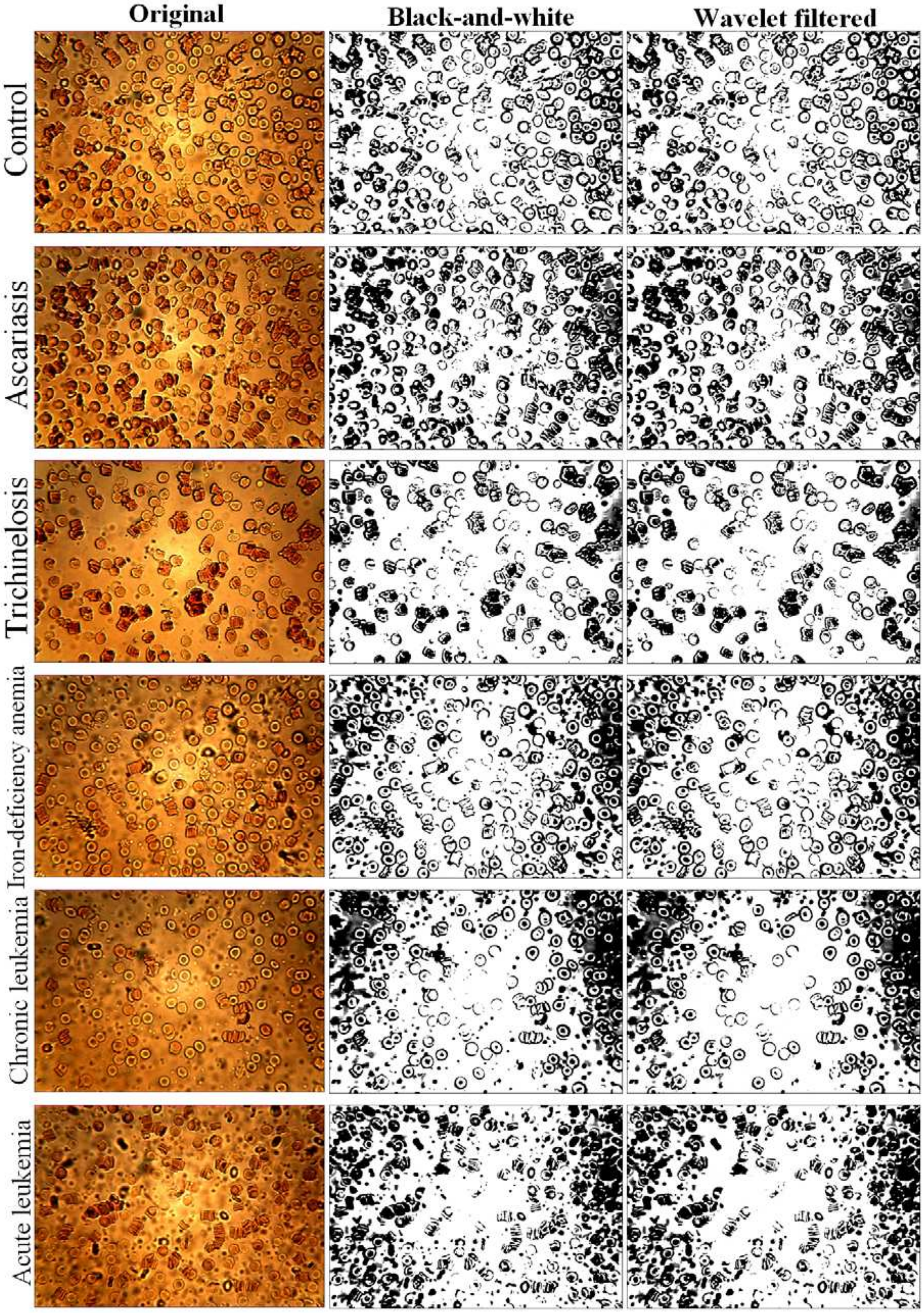

Figure 2. Images of control RBCs, Ascaris lumbricoides treated RBCs, Trichinella spiralis treated RBCs, iron-deficiency anemic's RBCs, chronic leukemic's RBCs and acute leukemic's RBCs at 40× magnification.

From a visual inspection, there are no dramatic changes among images within a column. However, the pictures from the third column, for which BCD values were calculated, showed significative differences in those values. Figure 3 shows the BCD for all the analyzed pictures, grouped according to the different disorders.

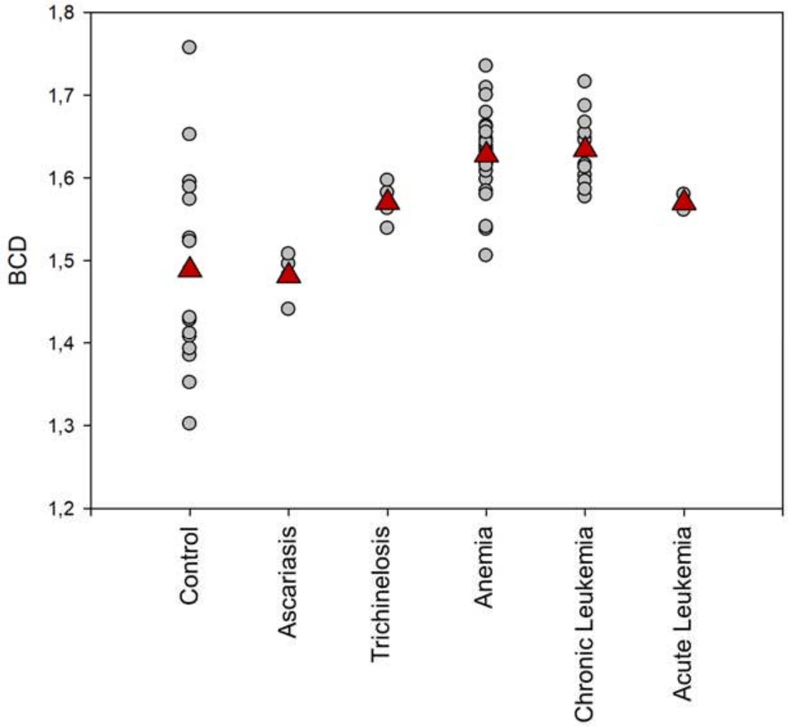

Figure 3. Box-Counting Dimensions obtained for RBC's control and treated samples.

In relation to the dispersion of the BCD values, samples corresponding to anemia, chronic leukemia and control showed a highly disperse pattern, in contrast to the observed dispersion for the other diseases. On the other hand, mean values are similar, though a separation in 3 groups could be proposed: control and ascariasis samples, trichinosis and acute leukemia samples, and anemia and chronic leukemia samples.

In order to statistically test these observations, we performed two non-parametric tests, as data did not pass the Shapiro-Wilk test. Levene's test showed that the variances corresponding to anemia, chronic leukemia and control samples were significatively different ($p < 0.01$) from the ones obtained from the other samples, confirming the previous classification. Dunn's test, however, allowed us to classify the samples in only two groups according to their BCD mean values ($p < 0.01$): one of them includes anemia and chronic leukemia while the other four diseases are included in the second group.

These results suggest that the methodology described could be used to differentiate among different blood disorders that affect RBCs, even when they did not generate evident visual alterations. Furthermore, it is useful to distinguish between different types of leukemia, suggesting that it is sensitive to the differential morphological changes that those generate on the blood cells.

4 CONCLUSIONS

The developed methodology has proven to be an effective and selective tool for clinical diagnosis of hematological relevant diseases. The chosen mathematical quantifier allowed a user-independent characterization to distinguish among different blood disorders. For diagnosis, it seems to be necessary to analyze a statistically significative number of photos, in order to obtain reliable diagnostic information. Moreover, this methodology does not require highly complex equipment, since the pictures can be obtained with a non-professional camera or even a cellphone, making it a viable alternative diagnosis tool in low-resources laboratories.

REFERENCES

Chien, S., Red cell deformability and its relevance to blood flow, *Annual review of physiology* 49(1): 177-192, 1987.

Grassberger, P. An optimized box-assisted algorithm for fractal dimensions, *Physics Letters A* 148:63-68, 1990.

Lupo, M., Leguto, A., Bortolato, S., Korol, A., Evolution of erythrocytes aggregation: A fractal approach when incubated with *Trichinella spiralis* and *Ascaris lumbricoides. Ain Shams Eng J* 2016 (doi: 10.1016/j.asej.2016.12.004).

Mancilla Canales, M., Leguto, A., Riquelme, B., Ponce-de-León, P., Bortolato, S., Korol, A. Hurst exponent: a brownian approach to characterize the nonlinear behaviour of red blood cells deformability, *Physica A* 488:1-7, 2017.

Ponce-de-León, P., del-Balzo, G., Riquelme, B. Biorheological action of *Ascaris lumbricoides* larvae on human erythrocytes, *Cell Biochem Biophys* 65(2):237-242, 2013.

Rapa A., Fractal dimensions in red blood cell, *Turk J Vet Ani Sci* 29 (6):1247-53, 2005

Talu, S., Stach, S., Kaczmarska, M., Fornal, M., Grodzicki, T., Pohorecki, Burda., W., Burda, K., Multifractal characterization of morphology of human red blood cells membrane skeleton *Journal of Microscopy* 262(1):59-72, 2016.