Asociación Argentina
de Mecánica Computacional

Mecánica Computacional Vol XXXV, págs. 2533-2538 (artículo completo)
Martín I. Idiart, Ana E. Scarabino y Mario A. Storti (Eds.)
La Plata, 7-10 Noviembre 2017

BOX COUNTING DIMENSION OF RED BLOOD CELLS SAMPLES WHEN FILTERED WITH WAVELET TRANSFORM

Ana M. Korol[a], Alcides J. Leguto[a], Juan P. Rebechi[a], Bibiana Riquelme[c], Patricia Ponce de León[b], Santiago Bortolato[a] y Manuel Mancilla Canales[a].

[a]*Departamento de Matemática y Estadística, Fac. Cs. Bioquímicas y Farmacéuticas de la Univ. Nacional de Rosario. Suipacha 531, Rosario, Argentina. korol@ifir-conicet.gov.ar*
[b]*Departamento de Parasitología, Fac. Cs. Bioquímicas y Farmacéuticas de la Univ. Nacional de Rosario. Suipacha 531, Rosario, Argentina.*
[c]*Instituto de Física de Rosario (IFIR-CONICET). Ocampo y Esmeralda, Rosario, Argentina, http://www.ifir-conicet.gov.ar*

Keywords: *box counting dimension, Wavelet Transform, red blood cells, Trichinella spiralis*

Abstract: Automatic recognizing of different populations of several millions of red blood cells (RBCs) is a useful tool in Hematology and Clinical Diagnosis. In this work we studied samples of several millions of RBCs: on one hand healthy control RBCs and on the other hand control RBCs incubated with *Trichinella spiralis* larval parasites. The alteration on the cells membrane with the parasite can be studied with box-counting dimension on both samples. Previously we applied wavelet transform to all the samples in order to improve the results. The procedure to remove noise from an image is based on the decomposition of the observed signal in a set of wavelets and taking threshold values to select the appropriate coefficients through which the signal can be reconstructed. In our work we compared the results obtained when analyzing the raw signals and the ones obtained after applying wavelet transform, and the results were different and more clearly characterized when the signal were treated with wavelet transform. Finally, the present method using wavelet transform is suitable to optimize the characterization of the RBCs damage when incubated with the larval parasites.

Copyright © 2017 Asociación Argentina de Mecánica Computacional http://www.amcaonline.org.ar

1 INTRODUCTION

Red blood cells (RBCs) are the most abundant cells in the blood and their primary function is to transport oxygen and carbon dioxide between the lungs and other tissues. Due to their mechanical properties and abundance, RBCs are also major determinants for the rheological behaviour of blood (Chien, 1987). In a previous work, our research group demonstrated how larval parasites interact with RBCs to generate erythrocyte aggregation, and how this interaction can be measured using a mathematical approach. Quantitative description of erythrocyte aggregation phenomena had been previously described, but with no conclusive results owing to the complex features of biological systems (Korol *et al*, 2013; Rapa, 2005; Talu *et al*, 2016).

Trichinella spiralis (Ts) is a pathogen parasite involved in the development of trichinosis. This disease is transmitted by food and is endemic in Argentina (Caracostantogolo *et al*, 2007). 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. The proposed analysis is based on fractal patterns measured at different portions of digital images of RBCs incubated with larval parasites.

In general, we could define a fractal as a geometric figure with very complex structure at any scale that is impossible to define by the concepts and methods developed since Euclides. This method applies Fractal Dimension (FD), a parameter that allows discrimination of different groups according to their nature; in our case, differentiation of healthy from ill RBCs. Fractal dimensions cannot be calculated accurately, but can be estimated. Although there are different definitions for Fractal Dimension (Mandelbrot, 1983), a widespread strategy to estimate it is box counting. Box counting can be applied to any distribution points, curves, surfaces, etc., and is the one chosen in our work, especially because we are dealing with pictures.

Wavelets are wave-like oscillation and their amplitudes are non-zero only during a short interval. Wavelet Transform (WT) is a time-frequency transform that decomposes a 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. Applications include data and image compression, partial differential equation solving, pattern recognition and noise/trend reduction (Aldroubi, 1996; Burrus *et al*, 1997; Unser *et al*, 1996).

In the present work, we use WT in order to have improved results. In particular, we found that WT could be used to reduce noise from images prior to applying the box counting method, allowing for modifications in FD values without sensitively affecting the images appearance.

2 MATERIALS AND METHODS

2.01 Samples preparation

Fresh group O blood samples were collected from a healthy donor into a container holding EDTA as anticoagulant, stored at 4°C and analyzed within 24 h. After removing autologous plasma, RBCs were washed three times with phosphate-buffered saline (PBS) (pH = 7.4; 295 ± 8 mOsmol/kg).

Infective larvae of Ts 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 0, 15 and 30 min at 37°C (treated RBCs). The RBCs used as control (control RBCs), were incubated only with PBS. After the incubation period, the treated and control RBCs were washed with PBS.

Copyright © 2017 Asociación Argentina de Mecánica Computacional http://www.amcaonline.org.ar

2.02 Digital image analysis

Treated and control RBCs samples were examined in a concave optical inverted microscope slide (Union Optical, Japan). Images for each sample (60 images analyzed) were obtained by duplicate (objective: 40X, Canon Powershot A640 digital camera) and were stored in JPG format for their further analysis. Images were then processed with the software Fractalyse. Fractal dimension was calculated according to an iterative principle based on the box counting method.

3 DATA ANALYSIS

3.01 Box counting

Box counting dimension is related to how information accommodates and is distributed in the image. In order to calculate it, an evenly spaced grid and a black and white image are needed. An approximation to box counting dimension is given by the following formula:

$$D(L) \simeq \frac{\log N(L)}{\log(\frac{1}{L})} \tag{1}$$

where ***L*** represents the side of the boxes, and ***N(L)*** the number of boxes required to cover the set (not empty boxes).

Finally, box counting dimension value comes from taking an infinite number of small boxes:

$$D = \lim_{L \to 0} \frac{\log N(L)}{\log(\frac{1}{L})} \tag{2}$$

3.02 Wavelet Transform

The process to remove noise from an image is based in the decomposition of the observed signal in wavelets 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 were used since they showed better results in the reconstructions of the images from their Inverse Wavelet Transform without altering the key features of the processed images.

4 RESULTS AND DISCUSSION

In figure 1, it is shown FD for the analyzed images at the three times of incubation with and without presence of Ts larvae.

Analysis of variance for FD in the absence of Ts larvae (figure **1.A**) showed no significant effect of time (p=0.79). This result is logical due to the absence of the parasite and at the same time confirms samples integrity throughout the incubation periods. A global mean of FD=1,6079 taking together all the observations could be obtained.

Analysis of variance for FD in the presence of Ts larvae (figure **1.B**) showed significant effect of time (p=0.0164). Then multiple comparisons (Fisher's least significant difference) were performed in order to identify different groups. It was found that the mean of the group at time 15 min was significantly different from the mean of the group at time 0. The group at time 30 min could not be established as significantly different from any of the groups.

Copyright © 2017 Asociación Argentina de Mecánica Computacional http://www.amcaonline.org.ar

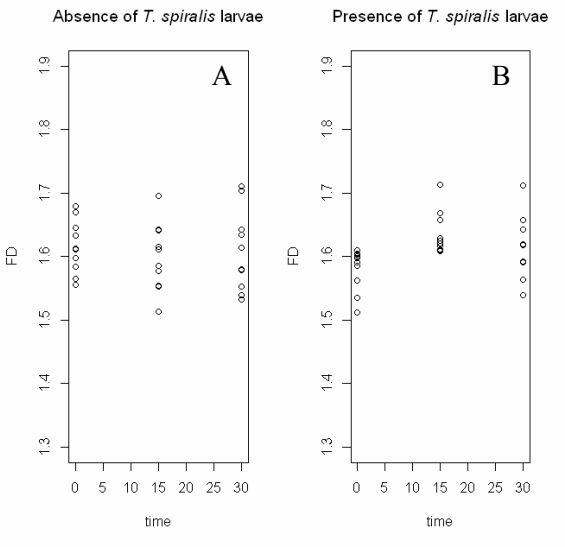

Figure 1: Fractal Dimension versus time of incubation (0, 15 and 30 min). A) In the absence of *T. spiralis* larvae (control RBCs). B) In the presence of *T. spiralis* larvae (treated RBCs)

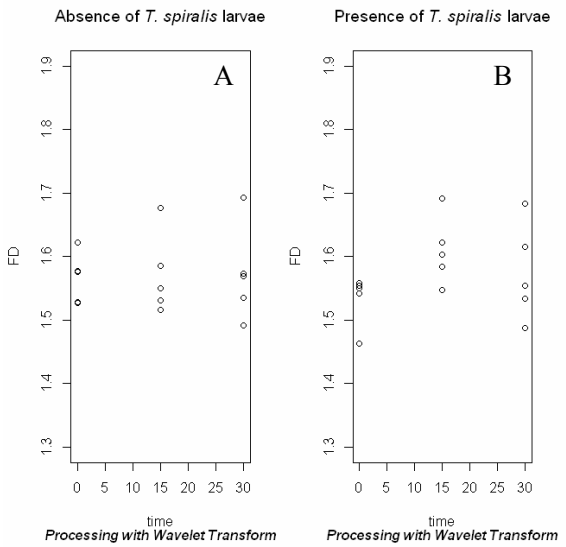

Figure 2: Fractal Dimension versus time of incubation (0, 15 and 30 min) after processing images with the Biorthogonal Wavelet Transform. A) In the absence of *T. spiralis* larvae (control RBCs). B) In the presence of *T. spiralis* larvae (treated RBCs)

In order to evaluate the incorporation of WT analysis in the study of the effect of Ts larvae in RBCs samples all the images were processed with Biorthogonal WT as described in materials and methods. The processing with the WT did not alter the underlying pattern in the data as can be seen from comparison of both figures.

The differences between values of FD before and after WT processing were calculated substracting the original FD value from post-processing one and are shown as a function of time in figure **3.A**. The mean value for all the differences was -0.0438 and was significantly different from zero ($p<<<0.001$).

The results suggest that after WT processing the FD decreases for all the FD values (figure **3.A**) and in this way the observed original pattern is maintained as all the values decrease on average 0.0438 (figure **3.B**). The difference between the analyzed images before and after WT is the presence of noise, so a plausible explanation could be that the new FD values after the WT better represent the fractal nature of the aggregations of RBCs determined through digital images.

Copyright © 2017 Asociación Argentina de Mecánica Computacional http://www.amcaonline.org.ar

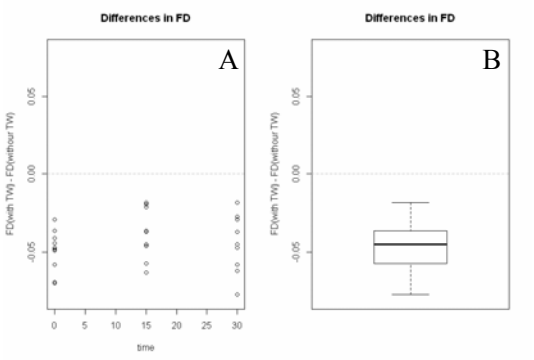

Figure 3: Differences in Fractal Dimension after processing with Wavelet Transform minus original Fractal Dimension. A) Versus time of incubation (0, 15 and 30 min). B) All the differences considered together.

5 CONCLUSIONS

In our work we compared the results obtained when analyzing the raw signals and the ones obtained after applying WT, and the results were different and more clearly characterized when the signal was treated with WT as we could evidence the maintenance of the underlying pattern of the data but with the advantage of noise elimination. The present method using the incorporation of WT was suitable to optimize the characterization of the RBCs alterations when incubated with the larval parasites.

The proposed approach of incorporating wavelet analysis and the use of a fractal quantifier does not pursue to replace conventional analysis but to incorporate a complementary view for the possible observable changes in RBCs integrity or aggregation through digital image analysis. The use of WT is a powerful tool for the analysis of biological images because of the complex nature of the samples and the search of a better WT that adapts more adequately to the pattern observed in the digital image is promising.

The incorporation of WT in the utilization of a computational technique (box counting) for the determination of FD of digital images of RBCs in the presence or absence of TS larvae attempt to generate new information, that could be available for the automatic recognition approach when working with different populations of RBCs in the hope of a future application in Hematology and Clinical Diagnosis.

REFERENCES

Aldroubi, A., Unser, M., Wavelets in medicine and biology, *CRC press*, 1996.

Burrus, C.S., Gopinath, R.A., & Guo, H., Introduction to wavelets and wavelet transforms: a primer, 1997.

Caracostantogolo, J.P., Steffan, J. Dillon; De la Sota, M.; Belgrano, D.; Veneroni, R.; Ruiz, M.; Schapiro, J.; Castaño, R.; Martínez, M., Mejoramiento del control de la trichinellosis en Argentina: Proyecto TCP ARG 3003 entre la FAO y el Gobierno Argentino. En*: Mejoramiento del control de la trichinellosis. FAO América Latina y el Caribe. Organización de las Naciones Unidas para la Agricultura y la Alimentación*, 3-66, 2007.

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

Copyright © 2017 Asociación Argentina de Mecánica Computacional http://www.amcaonline.org.ar

Korol E.A., Herrera I.M., Lamónaco A.N., Leiva Viú, N., Cantalejo Ceccato, M., Ponce de León P., Evolución de la dimensión fractal y correlación temporal de agregados eritrocitarios producidos por *Ascaris lumbricoides*. *Rev Cubana Med Trop* 65(3):283–96, 2013

Mandelbrot B.B., The Fractal Geometry of Nature. *San Francisco: WH Freeman*; 1983.

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, W. & Burda, K., Multifractal characterization of morphology of human red blood cells membrane skeleton. *J Microsc*; 262(1):59–72, 2016

Unser, M., Aldroubi, A. A review of wavelets in biomedical applications. *Proceedings of the IEEE*, 84(4), 626-638, 1996

Copyright © 2017 Asociación Argentina de Mecánica Computacional http://www.amcaonline.org.ar