



Enhancement of the robustness on dynamic speckle laser numerical analysis



R.R. Cardoso ^a, R.A. Braga ^{b,*}

^a Brazilian Bioethanol Science and Technology Laboratory, Brazil

^b Department of Engineering, Federal University of Lavras UFLA, CP 3037 Lavras, MG Brazil

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ABSTRACT

When a dynamic process occurs in a material under laser illumination the phenomenon that appears is named dynamic laser speckle, or biospeckle laser (BSL) if we have a biological material. The work with biological material and its dispersion of light brings considerable complexity, and the way we can deal with that complex outputs is based on a sophisticated analysis of the images associated to statistical approaches. One of the most known numerical analysis of the BSL has been applied in many applications, and it is named Inertia Moment, however its outputs have great coefficients of variation, most of the time attributed to the variability of the biological material. A change in the inertia moment method was done and the Absolute Value of the Differences (AVD) was presented as an alternative to reduce the variations and to follow a broader range of frequencies than before. However, it was not enough concerning with the variability of the outputs. This study aimed to improve the BSL technique in order to enhance the robustness of the numerical method known as Inertia Moment (IM) and improve the absolute value of the differences reducing even more its coefficient of variation by means of changes in the normalization provided in both methods. The new normalization was tested in simulated data, as well as in real data. The results showed the improvements of the methods, IM and AVD, with the reduction of the coefficients of variation of the activity in the outputs, increasing the robustness of the analysis.

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1. Introduction

Dynamic laser speckle is an optical phenomenon formed when an object in dynamic activity is illuminated by light with high coherence, for instance a laser. The coherence demanded has to be enough to provide a speckle pattern with clear definition of the grains. Therefore, the works using the biospeckle laser should adopt a laser of scientific purpose with high spatial and temporal coherence, which means the ability to maintain the speckle pattern stable when the scatterers do not move. The activity observed by the dynamic laser speckle in biological materials can be attributed to many features, such as growth and cell division, cytoplasmic movement and biochemical reactions, besides water-related activities, and changes in the microscopic structure of the illuminated sample [1]. Such changes make the interference pattern, formed by scattered light to vary in accordance to the intensity of the biological activity allowing the optical

monitoring of many sensitive phenomena in biological and even in non-biological samples [2].

Dynamic laser speckle, also named as BSL, is an optical approach that has advantages as it is a non-invasive, a non-destructive and a low cost technique for assessing and monitoring sensitive biological properties. Thus, BSL application has grown in optical instrumentation over the years [3], especially in some areas such as in medicine [4,5] and agriculture [1,6–9] and in non-biological samples such as the classic applications in coatings and paint drying [10,11].

One of the methods adopted in the BSL analysis presents numerical results with a great variability that initially was attributed to the variability of the biological material. The Inertia Moment (IM) [12], as the numerical method is known, was questioned in some applications [13], and it received an alternative procedure in order to reduce its variability and to consider the low frequencies in the BSL signal during the numerical analysis. The alternative procedure was defined as the absolute value of the differences (AVD) [14,15]. The IM method carry out the analysis of the time history of the speckles, and in one of its steps it computes the square difference of the points in a co-occurrence matrix, which represents the variation of the signal. In turn, the AVD provide the absolute value instead of the

* Corresponding author.

E-mail address: robbraga@deg.ufla.br (R.A. Braga).

square value of the differences of the points in the same co-occurrence matrix. Despite the reduction of variation and the best consideration of the low frequencies [14,15], the AVD method can receive improvements in order to make it more robust.

The numerical analysis of the BSL can be also conducted using many other approaches such as contrast of each speckle pattern [4,16], or autocorrelation of the time history of the speckles [8,13,17–19]. The adoption of one of the multiple methods as a “gold standard” in the field is still a challenge, which addresses the demand for improvements and comparisons of the known methods.

Therefore, this work aimed to present an adjustment of the numerical method of analysis of BSL. The numerical outcomes of IM and AVD were changed in order to reduce the variability present in the prime normalization occurred during the analysis of the data, caused by the inhomogeneities in the speckle images that are common in many applications.

2. Theory

2.1. Inertia moment's normalization

The use of the IM method as a tool to quantify the biological activity by means of the time history of the speckle patterns has been used in many applications since its proposal [12]. Regardless of its reference in the field, the IM approach presents some limitations and the first, in our point of view, is the great variation of its values even when the phenomenon does not present that expected variation.

One tentative work to deal with that variation and to reduce it in the outputs was the proposal of the Average Value of the Differences (AVD) which enhances information of biological activities that also occurs in low frequencies in the time history of the BSL, and reduced the great effect of the high activities [14,15]. Eq. 1 presents the IM method with the square of the differences that was substituted by the absolute value in the AVD.

$$IM = \sum_{i,j} M_{ij}(i-j)^2 \quad (1)$$

where M is the normalized co-occurrence matrix (COM) [12] in coordinates i and j . The conventional normalization proposed [12] is shown in the following equation:

$$M_{ij} = \frac{COM_{ij}}{\sum_i COM_{ij}} \quad (2)$$

where COM_{ij} is the value of the co-occurrence matrix in a row i and a column j . Thus, normalization makes the sum of values in each row of the COM equal to one. The prime IM method [12] is based on the construction of a COM [20] from the time history speckle pattern (THSP) [17,18]. Despite the adoption of the COM in many areas, its normalization was only carried out in the IM method [12], and without any identified reason.

Therefore, the normalization proposed by the prime method [12] provides a normalization that considers the whole image as homogeneous, correcting each line of the COM as presented in Eq. 2. In turn, the proposed method fixes the normalization with a number that represents the summation of all occurrences in the

COM, according to the following equation:

$$M'_{ij} = \frac{COM_{ij}}{\sum_{i,j} COM_{ij}} \quad (3)$$

3. Methodology

The validation of the hypothesis related to the improvements of the inertia moment and of the absolute values of the differences linked to the normalization of the occurrence matrix was divided in three parts. The first validation created some hypothetical THSPs and tested them using the two normalizations. The second test of the normalizations were simulated in a real data, which means the use of a real THSP with the introduction of fake changes in the grey levels, and the third validation was provided by the analysis of real data comparing the different approaches of normalization and the dispersion of the numerical outputs.

The first test was conducted by means of an example, constructing a fake THSP and co-occurrence matrices to explain the influence of the variation of brightness in the two normalizations. The three hypotheticals THSP were constructed in order to understand the practical difference and consequence of using conventional/proposed normalization. To make it didactically, these THSP were constructed considering a size of 5×8 pixels and resolution of 2 bits.

These THSP can be seen in Fig. 1, where each square represents a pixel from hypothetical THSP, and they have very different grey level distribution as shown in Fig. 2. The second test was carried out by means of an intervention in a real data, particularly adding a negative offset in the grey scale values in a delimited area of the Time History Speckle Pattern matrix thus simulating a dark spot in the image caused by, for example, the curvature of the sample or even by some irregularity in the illuminated surface. The test was conducted in a THSP from an animal sperm, considered a homogeneous sample, which means the absence of curvature or irregularities that could cause some shadows in the images. An offset reducing the brightness was introduced in the THSP, that it was provided decreasing 50 points in the level of grey in the first 100 lines of the THSP image (512×512) as presented in Fig. 3. The negative values, after the subtraction, were fixed in zero, in order to maintain the 8 bits format.

The occurrences of values equal to zero caused by the subtraction of 50 from the area of the THSP were despised, since the amount of zeros added in the image changed from 0.04% to 0.4%. The amount of zeros changed from 128 to 1101 after the subtraction of 50 points in the first 100 rows of the image of Fig. 3a. The sub-exposition of the images could cause some false answers in the IM and AVD analysis [21], however that was not the case, since the amount of values equal to zero introduced is still low (0.4%) in the whole image.

The reduction of illumination was based on the relation between the intensity of the incident light (I_i) and the intensity of the light that returns to the observer (I_r).

$$I_r = \mathcal{R}I_i \quad (4)$$

where \mathcal{R} is the reflectivity index.



Fig. 1. Hypotheticals THSP with (a) high occurrence of dark pixels, with (b) the grey levels randomly distributed, and with (c) high occurrence of light pixels.

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