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Biological feature isolation by wavelets in biospeckle laser images

R.A. Braga Jr.[a,*], G.W. Horgan[b], A.M. Enes[a], D. Miron[a], G.F. Rabelo[a], J.B. Barreto Filho[a]

[a] Departamentos de Engenharia/Veterinaria, Universidade Federal de Lavras, Lavras, CP 3037, CEP 37200-000, MG, Brazil
[b] Biomathematics & Statistics Scotland, Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK

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Abstract

Biospeckle analysis has been used as a tool to analyse biological activity with successful results in areas such as fruits, seeds and animal reproduction. These results use well-known image analysis methodology to treat the data with a second order statistical approach. Further development of this technology requires the use of new approaches to process the data and get more information, in order to make the methodology more robust and reliable. This paper presents the results of analysing biospeckle data using wavelet transformations, coefficient filtering and reconstruction, with a novel approach to increase the information obtained. The data used were from two distinct biological investigations, crop seeds and animal sperm, and the results showed that the wavelet transform was able to identify the effect of dilution in the sperm sample, allowing the isolation of nuisance covariates. The reliability of the methodology allows the development of a powerful tool to understand the influences on the patterns in the biospeckle data, even in complex materials like those observed in seeds.

Keywords: Laser; Dynamic speckle; Wavelets

1. Introduction

Biospeckle laser technology has been proposed as a powerful tool to obtain information from biological material by Briers (1975, 1993), Asakura and Takai (1981), Oulamara et al. (1989), Xu et al. (1995) and Ross et al. (1983). The name biospeckle is a derivation of the term 'dynamic speckle', in accordance with Asakura and Takai (1981), which is also referred to as 'speckle fluctuations' by Briers (1993) and Ross et al. (1983). It results from rapid changes in the laser interference pattern caused by microscopic changes due to cell activity in living material. Inert material will show no speckle changes. Biospeckle therefore offers a relatively easy method of measuring the level of biological activity in samples.

One approach to analyse the images from laser illumination of tissues consists of creating a space time speckle (STS) pattern, as suggested by Oulamara et al. (1989) and Xu et al. (1995), also known as a Time History of Speckle Pattern (THSP) (Arizaga et al., 1999, pp. 163–169). With the STS it is possible to estimate the degree of activity of an illuminated object on the basis of its dynamic speckle behaviour. Most approaches described in the literature have been based on examining a single summary of the speckle pattern. The value of the summary of the speckle pattern could be derived from an auto-correlation curve, for example, or from the co-occurrence matrix, such as the inertial moment (IM) presented by Arizaga et al. (1999).

* Corresponding author.
E-mail address: robbraga@gmail.com (R.A. Braga Jr.).

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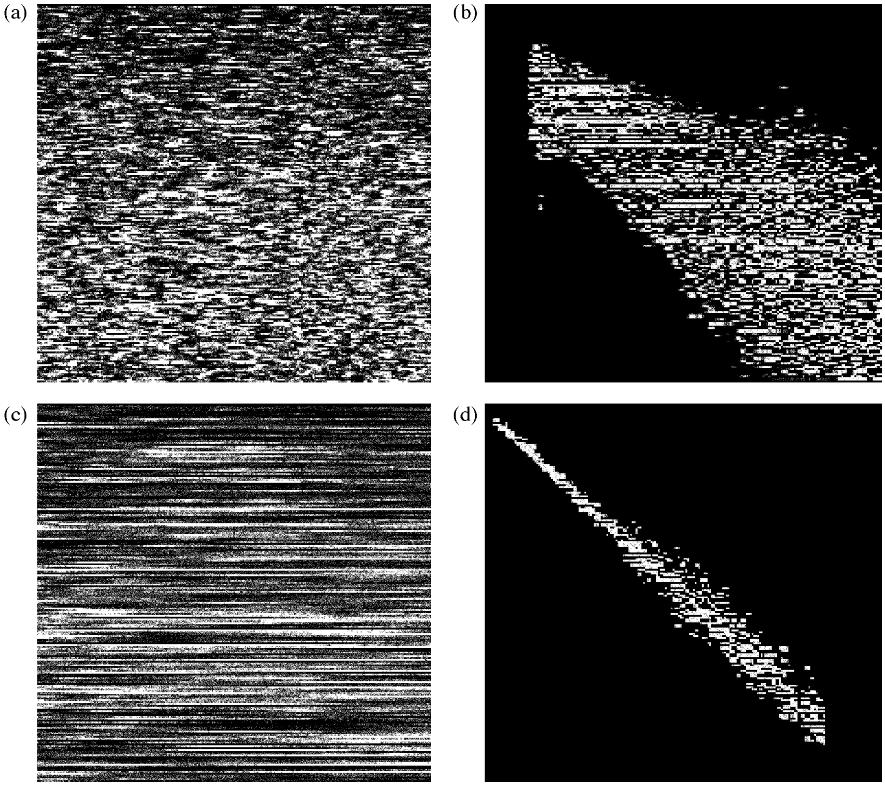

Fig. 1. (a) STS pattern, 512 × 512 pixels, of a seed with a high level of moisture; (b) co-occurrence matrix, 256 × 256 grey scales, from a wet seed STS; (c) STS pattern, 512 × 512 pixels, of a seed with low level of moisture; (d) co-occurrence matrix, 256 × 256 grey levels, from a dry seed STS.

The dimensions of the data may vary, and it is possible to use any size from just 1 pixel to, for example, 512 pixels. Approaches to the analysis of the information in the STS patterns also vary, with autocorrelation used in Xu et al. (1995) and the inertial moment IM proposed by Arizaga et al. (1999).

The IM values are obtained from the co-occurrence matrix that is defined as

$$\mathrm{OCM} = [N_{ij}] \tag{1}$$

where N_{ij} is the number of occurrences of a grey level i followed by a grey level j in the STS matrix, and the OCM is a 256 × 256 matrix (in the usual case of 256 intensity levels). The concept of OCM has been presented in a different way by Park et al. (2002). The inertial moment is defined as

$$\mathrm{IM} = \sum_i \sum_j \left(\frac{N_{ij}}{\sum_j N_{ij}} \right) \cdot (i-j)^2 \tag{2}$$

Here the summation is over the intensity levels i and j. Fig. 1 shows two STS patterns with their respective co-occurrence matrices (Braga et al., 2003, pp. 287–294). The matrices plotted are from two seeds with a distinct moisture level.

Although biospeckle technology has presented reliable results, e.g. for seeds (Braga et al., 2003, pp. 287–294; Braga et al., 2005, pp. 465–469), animal sperm (Nascimento, 2005), fruits (Rabelo, 2000; Pajuelo et al., 2003, pp. 13–24) and soap films (Tebaldi et al., 2004, pp. 29–37) some information is lost during the procedure of getting just one number to summarise the behaviour of the process. This can be a limitation when the object analysed presents changes over time, or when the data present a composition of features in distinct frequencies (Jakubauskas et al., 2002, pp. 127–139; Yang et al., 2005, pp. 255–271).

One technique that has been used to deal with non-stationary time variation pattern is the wavelet transform as presented by Alsberg et al. (1997), Torrence and Compo (1998) and Zakharov and Zimnyakov (2001). The wavelet