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Biospeckle numerical values over spectral image maps of activity

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ABSTRACT

The image analysis presents itself as a powerful instrument applied to all sort of biological phenomena monitoring. The development of many optical approaches to carry out a feasible image assembling and analysis to different demands has been the main effort in this application area. A consequence of that effort is the adoption of the biospeckle laser technique as a potential alternative to pursue the optical metrology. Particularly, the monitoring of the biological activity under the laser illumination presents as a reliable tool to many applications in many areas, such as to identify the changes in the micro-blood flow in animal tissues, or even to monitor the vegetal and the animal tissues and their metabolism. However, one limitation of biospeckle is the access of graphical maps of activity with any numerical information linked to them. This work had the objective to present a protocol to separate different tissues in the same material by means of the frequency signature, and by means of the association of graphical and numerical results from the biospeckle laser images. In order to confirm the efficiency of the proposed protocol we applied it to separate embryo and endosperm in maize seed and as well to separate tumour cells and normal tissues in animals. The results showed the feasibility of the approach proposed offering results with graphical maps associated to numerical information.

1. Introduction

The application of dynamic laser speckle, or biospeckle laser BSL, in many areas of knowledge created naturally new demands of research and developments [1–4], but always carrying out the results with the separation of the graphical to the numerical approaches. In medicine the adoption of biospeckle laser has been widely used where a capillary blood flow in the human skin is present [5–7], every time separating the graphical to the numerical approaches. Many applications in particular linked to the Doppler perfusion phenomenon and as well as to contrast technique were registered using graphical approaches [8–12]. Despite the growing usage of dynamic laser speckle in blow flow phenomena, the use of biospeckle laser in tissues without a well defined flow was considered a more complex approach [13]. The development of technologies associated with the dynamic laser speckle has offered new alternatives to access the sensitive activities in animal and vegetal tissues [3], in particular in those with a non-defined flux [14] the ocular microtremor was evaluated.

The research of cancer identification, in turn, demands huge and permanent efforts to scientists such as to study the metastases [15]. The optical techniques are an actual alternative to achieve the diagnosis of tumours which are also known as optical biopsy [16,17]. The use of biospeckle in cancer cell detection can be observed in the literature [18] where the biospeckle and the Stokes vectors were adopted to early diagnostics of connective tissues pre-cancer states. In addition, a way to detect malignant melanoma by laser speckle and contrast technique with numerical output was proposed [17] but without the generation of activity maps associated to numerical values. It is observed as well that the effort to achieve the cancer cell identification was also proposed in the frequency domain, in particular using the hyperspectral imaging [19]. In turn, the frequency domain has been one of the alternatives to achieve and enhance areas of different activities using biospeckle [20]. Differentiation of a low activity area inside the same tissue with high activity, like damage or fungi in seeds, is one challenge for the researchers. The study of seeds analysis in frequency domain in order to overcome the difficulty to isolate low activity areas has been presented [21–24] searching for spectral signatures for the phenomena linked to seed activity.

This work aimed to present an approach to obtain numerical values for biospeckle phenomena within graphical maps of activity

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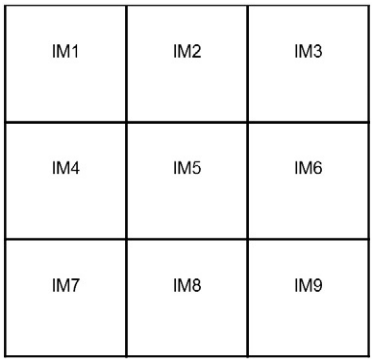

Fig. 1. Window of interest (IM5) and its neighbourhood windows (IM2, IM4, IM6, and IM8) for homogeneity calculation.

by means of frequency domain to create signatures to different activities, in particular linked to different cancer and seed tissues.

2. Material and methods

The methodology adopted to evaluate the cancer cell identification was based on an image approach, in particular, using the Fujii Method [7] before and after the frequency decomposition (Eq. (1)).

$$Fuji(x,y) = \sum_{k=1}^{N} \left| \frac{I_k(x,y) - I_{k-1}(x,y)}{I_k(x,y) + I_{k-1}(x,y)} \right| \quad (1)$$

Where the processed image, *Fujii(x,y)*, represents the differences between the image I_k and the image I_{k-1} in each pixel (x and y).

The frequency decomposition was carried out by the wavelet transform which was applied to each pixel of the 128 images (640×486 pixels) for the cancer tissues and 64 images (256×490 pixels) for the seed images assembled in time, as presented in Eq. (2) with the wavelets coefficients being represented by *CWT(t, j)*, as a function of time (t) and scale (j), from the signal in time *f(t)* being convolved with the Wavelet mother Morlet [25].

$$CWT(t,j) = f(t) * W(j,t) \quad (2)$$

After the decomposition of each pixel in time, the reconstruction of the collection of images was based on 25 and 21 scales of frequencies for cancer and seed tissues respectively from the wavelets

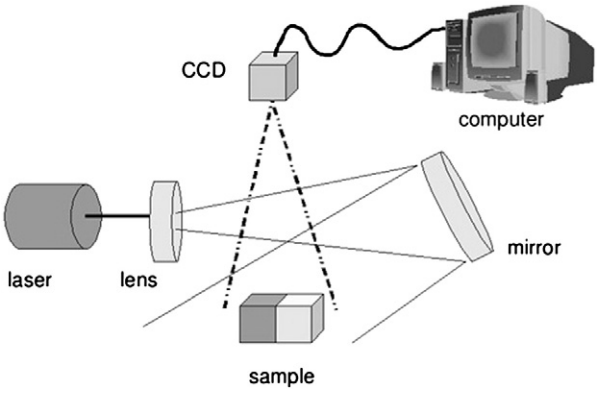

Fig. 2. Backscattering experimental configuration of tissues illumination and image acquiring.

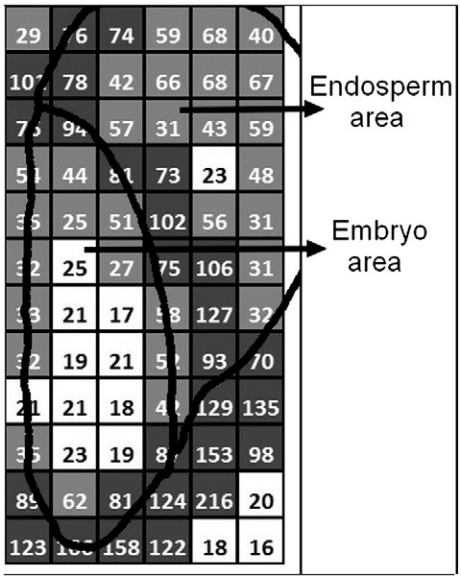

Fig. 3. Homogeneity values distribution in maize seeds using the IM.

transform as presented in the Eq. (3) [25]. The number of frequency ranges varied in accordance to the number of images assembled [26].

$$f(t) = K \sum_{j} \Re\{W(j,t)\} \quad (3)$$

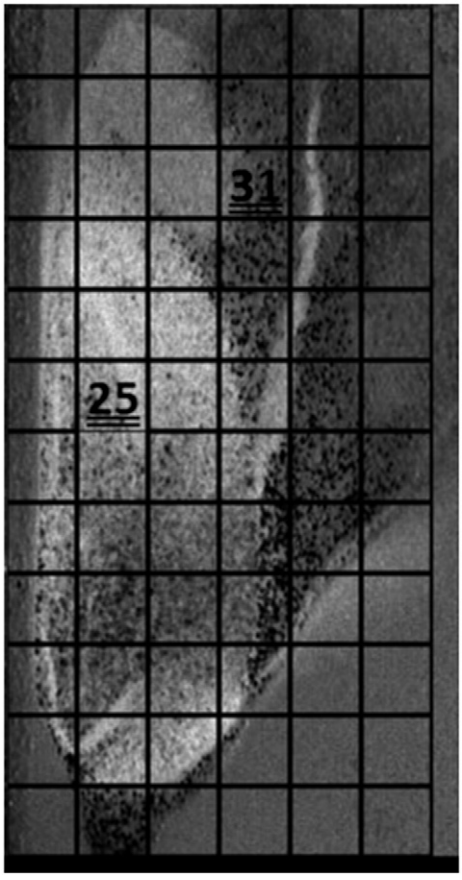

Fig. 4. Extracted homogenous areas in Fujii treatment.