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Challenges to Apply the Biospeckle Laser Technique in the Field

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Biospeckle laser, or dynamic laser speckle, is used as a technique when its outcomes are useful to monitor activity of the illuminated sample. The term “bio” means the relation of the activity with biological material, and the adoption of the dynamic laser speckle has been reported since 1970s, initially in blood flow monitoring. The range of applications improved along the years from medicine to agriculture, food and other areas. However, despite the increase of applications, there are some limits or challenges that are necessary to overcome in order to make this technique useful far from the optical laboratories. The instability of the solid-state lasers, used to reduce the size of the setup, as well as the influence of the external light and vibration can compromise the results and are some examples of the limits found. The standardization of the procedures to maintain the repeatability is another issue that must be faced, associated to the portability and accessibility of the devices. This work presents the state of the art in the area of biospeckle laser application and discuss the efforts that have to be taken to make it accessible, robust and reliable. A proposal of a free library in M code (Matlab and Octave) with its tutorial is presented and shared as a way to start the standardization of the image analysis. The library has a collection of numerical and graphical codes, as well as frequency filters to isolate phenomena. A list of applications is presented associated to the advances regarding the new born experimental configurations, mainly related to the digital devices used to acquire and to analyze the data.

1. Introduction

The Biospeckle Laser (BSL) is a dynamic interferometric phenomenon (Rabal & Braga 2008), which has been adapted as a sensitive tool to monitor changes in biological samples, and thus it has been applied in many areas, from medicine (Briers 1975, Asakura 1988) to agriculture (Xu et al. 1995, Zdunek et al. 2014), since the fact that it is a Non-Destructive Technique (NDT) is relevant in biological applications.

The multitude of applications is associated to a range of methods to illuminate, to assemble the images and to provide their analysis. The applications in agriculture offer the potential monitoring of alive phenomena by a NDT approach, in a sensitive way, overcoming the time consuming and the human dependence of judgement. The identification of activity in seed analysis (Braga et al. 2003), the study of the growing process in roots (Braga et al. 2009) or even the monitoring of maturity of bruising in fruits (Xu et al. 1995, Pajuelo et al. 2003, Kurenda et al. 2012) are some examples of feasible applications.

The static appearance of a speckle pattern is expressed by an image with clear and dark grains distributed over all the illuminated material as presented in Figure 1, where a tissue of a root growing in a gel, in this case, magnified in one portion of the root to be possible to observe the grains. Since it is a dynamic phenomenon, the speckle pattern expresses the boiling effect with the grains changing their shape and level of lightness related to the level of movement of the scatterers of the coherent light. The scatterers in an inert material, for example, do not change, so much so, the boiling effect isn’t observed, whilst the boiling is observed in the tissue of the root, and its intensity is linked to the level of activity.

One outcome of the BSL is the map of activity, as observed in Figure 2, where the Generalized Difference method (Arizaga et al. 2002) was applied. Thus, the map of activity that can be displayed in pseudo-colour located in the areas where one has more activity of the scatterers in time, in red, and where one has low activity, in blue. From blue to red it is possible to tag the level of activity and with it classify the biological phenomena that caused the boiling effect. Since a biological material is very complex with a multitude of

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phenomena occurring at the same time, in most cases it is impossible to isolate directly the main cause of the dynamic effect. Despite the feasible application in seeds, fruits, roots, as well as in animal sperm (Carvalho et al. 2009), parasites (Pomarico & DiRocco 2004), and many others in agriculture, the main limitation so far is the restriction to optical laboratories. The main aim of this work is to identify the challenges to use the biospeckle phenomenon in the field facing all sort of interferences suggesting some alternatives to overcome the limits.

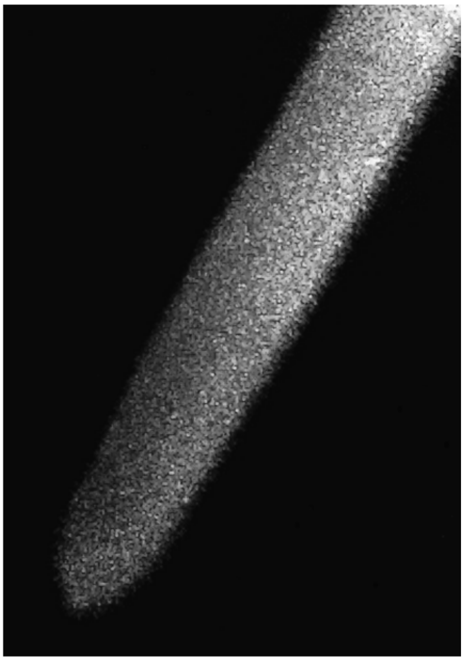


Figure 1: Outcome of an illumination of a sample using the laser, with the grains representing the speckle, amplified to magnify the grains. Raw data of root of coffee from Centre for Development of Applied Instrumentation in Agriculture (CEDIA)

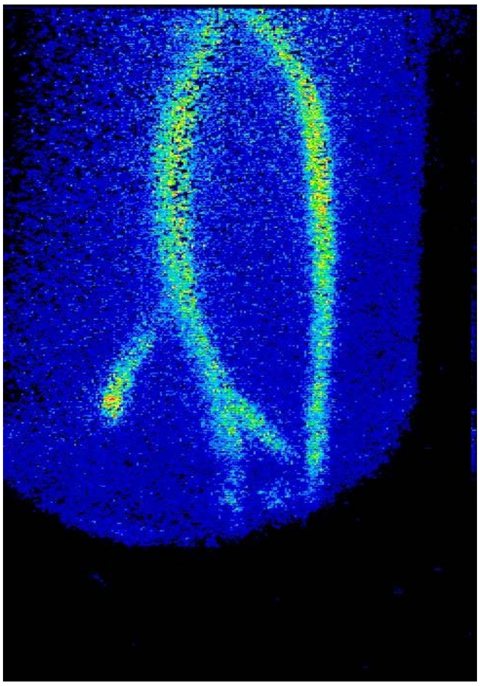


Figure 2: Outcome of the dynamic image of speckle in time after graphical processing with the map of activity. Raw data of root of coffee from Centre for Development of Applied Instrumentation in Agriculture (CEDIA)

2. Electing the limits

The absence of a standard, and even of a commercial equipment dedicated to agriculture to create some common approaches can be considered the main limit to the biospeckle accessibility as a technology to measure biological activity.
However, the development of digital electronics and laser sources opened new doors regarding the biospeckle laser phenomenon and certainly the possibilities to build reliable equipment. New cameras, computers and lasers did a revolution in the applications as well as in the field of image and signal analysis. The migration to the usage of biospeckle phenomenon in the field, such as measuring water activity in leaves without tear them, or the presence of biological material in a soil must overcome the limits regarding placed by the use outside doors with light and noise interference.
The light interference can be circumvented using dark environment around the laser and the digital camera. While the noise can be circumvented by the adoption of fast measurement techniques, such as the ones adopted in blood flow monitoring in alive human beings (Briers & Webster 1996, Godinho et al. 2012). Fast measurement means the measure using only one image or a short collection of image faster enough to avoid the mechanical noise interference.
In Figure 3, it is possible to gather some challenges that can be addressed to enhance the hardware and software allowing the portability and robustness needed.

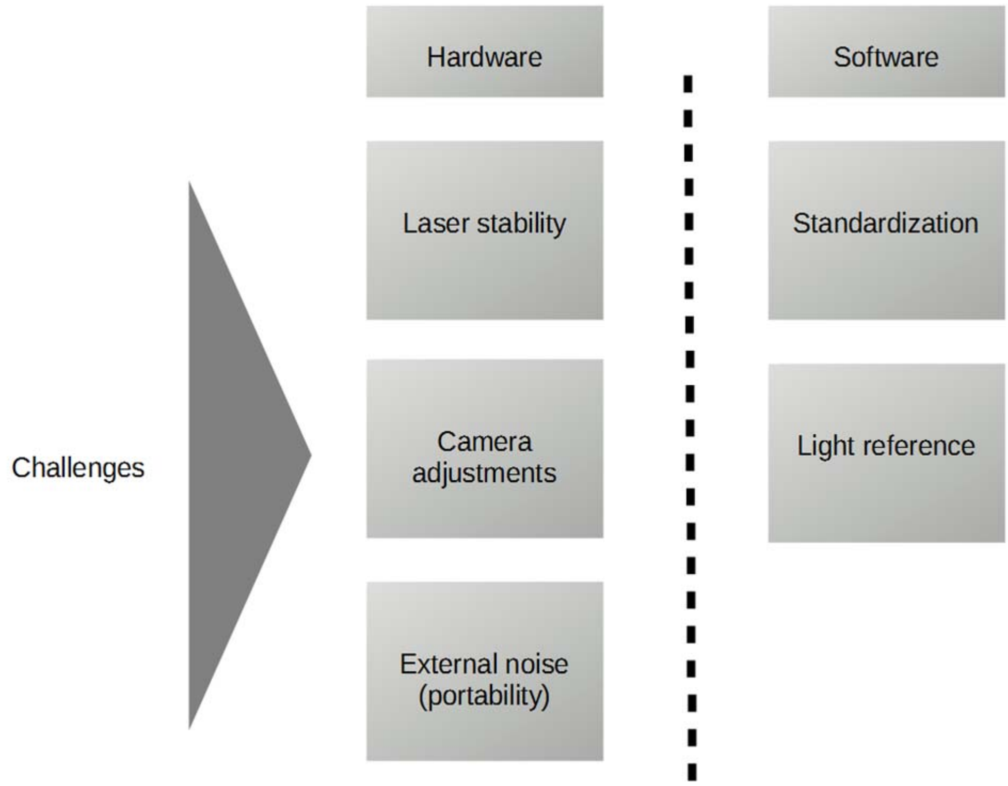


Figure 3: Scheme of the challenges driven by the limits to use the dynamic laser speckle regarding hardware and software

3. Discussing the limits

During many years, the applications of the dynamic laser speckle phenomenon, particularly, in biological material, became familiar (Zdunek et al. 2014, Rabal and Braga 1998) as well as the limits of its usage. In Figure 2, the limits were divided in hardware and software and the discussion about those limits are addressed in the next subsections.

3.1 Laser stability

Traditionally, the laser most used in this sort of interferometric measurements is the HeNe laser, however there is a great limitation to move it away from the optical laboratories. In addition, the variety of wavelengths offered by diode lasers is high. The adoption of diode laser has been useful in many applications, since its reduced costs and size, however the stability of the laser can be compromised by, for example, the low quality of the power source or non-stable operation of the laser set (Ahmed et al. 2001, Ahmed 2003). Therefore, the use of diode lasers must be carried out after tests of stability of the laser.

3.2 Camera adjustments

There are many options of cameras in the market, however the user must have in mind that the parameters of the camera, such as the zoom, the ISO, the white (light) balance as well as the speed can be in automated set point as a factory default, which means that the camera will continuously bias those parameters in accordance with the changes in the illuminated objects and that can compromise the result of dynamic phenomenon such as the speckle laser (Braga et al., 2016). Thus, a mandatory step before the assembling of the data is the setup of the camera disabling the automated adjustments. In doing so, the user will be able to apply cheap cameras. Additionally, the adjustment of the ratio of the system's focal lens to the diameter of the entrance pupil (iris) named f-number. (González-Peña and Braga, 2016)

3.3 Portability

The use of dynamic laser speckle out of laboratories dedicated to optics can be seen as the next step of its use, however it is necessary to circumvent the limits presented by mechanical noise, by external light and by the needed robustness of the apparatus. For example, the power supply must be independent and therefore must be designed to have long live and avoid ripples. The system proposed to measure parasites can be an example of portable equipment using the forward scattering approach (Pomarico & DiRocco 2004).

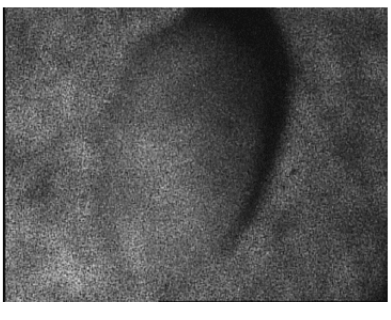
3.4 Standardization

The presence of many approaches to analyse the phenomenon and provide a reliable outcome need to be gathered and the alternatives compared, in the same way, they should be classified and organized. One can see that there are online and offline approaches, where the online are restricted to two methods: Laser Speckle Contrast Analysis (LASCA) (Briers & Webster 1996) and the Motion History Image (MHI) (Godinho et al. 2012) and the main outcome is graphic.

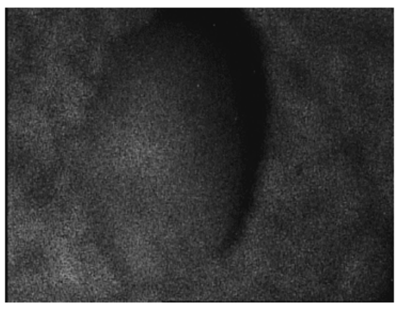
In turn, offline procedures to analyse the dynamic laser speckle data can be divided in time and frequency domain, and in both cases the possible outcomes can be numerical or graphical. All these known methods, so far, were gathered in an open library (Braga et al. 2016) to encourage the standardization of the procedures. The library was constructed in M Code related to MATLAB and Octave platforms, with free access of raw data of dynamic laser speckle (http://www.nongnu.org/bsltl/).

3.5 Light reference

The influence of the light during the dynamic laser speckle must be considered appropriately since the change of the level of the light can cause variations in the final outcomes (Reis et al. 2016). The methods adopted to analyse the raw data can be adapted to circumvent the light variation, however if the amount of light is insufficient to bring the needed information from the biological material, there is no way to circumvent this lack of light. The user must have in mind that the level of light plays a key role in the dynamic laser speckle as can be seen in Figure 4, where the same seed was lit by the same laser with distinct intensity. The histogram of the distribution of the grey level intensity can be seen in Figure 5, clearly showing in bold the distribution of grey in a seed with sub-illumination.



(a)



(b)

Figure 4: Level of illumination using the laser in a coffee seed with (a) high level of light and with (b) low level of light. Raw data of coffee beam seed from Centre for Development of Applied Instrumentation in Agriculture (CEDIA)

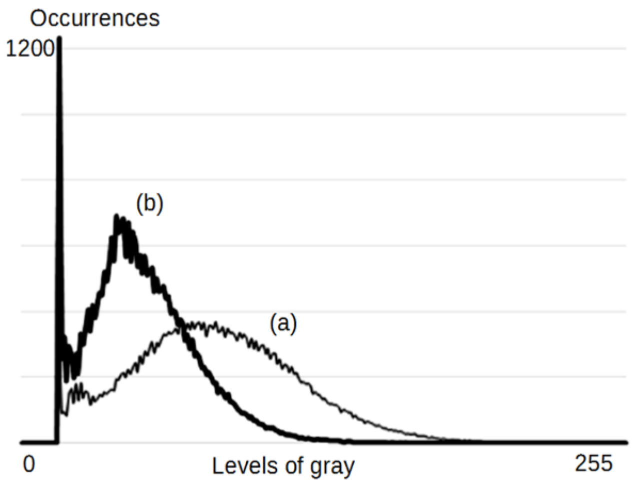
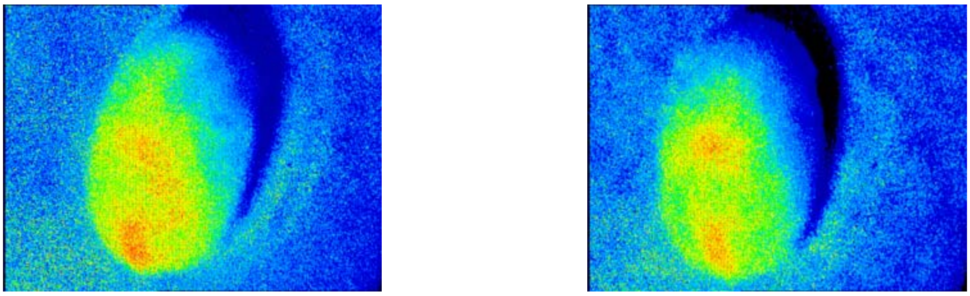


Figure 5: Histograms of the two images of a coffee seed with (a) high level of light in continuous line and with (b) low level of light in continuous bold line

The result of the sub-illumination of the seed can be observed in Figure 6, after the processing using the standard deviation analysis (Cardoso & Braga 2014), where the map of activity, presented in pseudo-colours (from blue representing low activity to red representing high activity), is shown with areas of high activity in the seed with more light (Figure 6a). Thus, many questions rise from the level of illumination and the most critical question is if the intensity of the laser wasn't enough to bring all the information we needed from the inner layers of a seed, that is a complex organism with many structures. If the light could not get through the desired layer, we will not be able to analyse the information of the scattered light from its elements.



(a) (b)

Figure 6: Standard deviation processing of illuminated coffee seed with (a) high level of light and with (b) low level of light (from blue representing low activity to red representing high activity). Raw data of coffee beam seed from Centre for Development of Applied Instrumentation in Agriculture (CEDIA)

4. Conclusions

This work presented the adoption of the phenomenon provided by dynamic laser speckle as a reliable tool to monitor the biological activity and the main challenges to its usage in the field. The challenges were divided in hardware and software, with the limits presented and commented. The presentation of a standardization project to circumvent the lack of a known protocol was done, and there was also presented the results of the influence of the level of the light in the experiments. The domain of the phenomenon and the knowledge of its limitation are relevant to help new users to avoid drawbacks in their applications. The standardization of the analysis helps the development of the technique and its usage by those who is not so keen to deal with software. This is a key step in the help the industry/academy to create equipment to the multitude of applications in agriculture, providing more instruments to enhance the economic sustainability of the agricultural processes.

Acknowledgments

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