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REVIEW

When noise became information: State-of-the-art in biospeckle laser

Quando o ruído tornou-se informação: O estado da arte do biospeckle laser

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ABSTRACT

Laser was presented to science and industry in the 1960s and shortly became a useful tool in many areas, with applications based on its multiple characteristics such as coherence of light, which presents a phenomenon known as interference pattern, or speckle, when beam returns from an illuminated surface. Despite great application of speckle pattern, its residual presence, for example, in interferometric approaches was considered as a noise, demanding filtering. However, grains themselves became information as their dynamic changes in time started to be linked to biological sample activity. Dynamic laser speckle has been since then a phenomenon widely used to monitor biological activities in many areas from agriculture to medicine. It is known as biospeckle laser (BSL) when adopted in biological material, with high sensitivity to follow very tiny movements in biological tissues, linked to changes in speckle provided by scatterer activities inside and outside cells. Since the 1970s, biospeckle laser usage follows a crescent technologic spiral where technological developments opened room for new applications, while new demands regarding biological monitoring forced the development of new methodologies. Therefore, potential adoption of the phenomenon as a sensor, for instance, in agricultural and medical processes, as well as constant offer of new devices provided new turns in the BSL technologic spiral and opened room for technique improvement. In this study, I present a short history of biospeckle laser (BSL) with applications and development associated with challenges regarding its usage in portable and accessible devices or even in commercial equipment. And the history was packed in a temporal diagram identifying the breakpoints responsible for improvements in the use of the technique.

Index terms: Optical metrology; biosystems; challenges.

RESUMO

O laser foi apresentado à ciência e à indústria em 1960, e rapidamente tornou-se uma ferramenta útil à muitas áreas do conhecimento, que fazem uso de suas múltiplas características, como é o caso da coerência da luz, que é responsável pelo fenômeno conhecido como padrão de interferência, ou speckle; este padrão de interferência ocorre quando a luz retorna do material iluminado formando uma figura de interferência para o observador. Apesar da grande aplicação, a presença residual do padrão de speckle em abordagens interferométricas apresentava-se como um ruído que demanda filtragem. Apesar da dificuldade em alguns casos, os grãos de speckle tornaram-se fonte de informação em situações dinâmicas, quando suas mudanças começaram a ser relacionadas à atividade do material biológico iluminado. Speckle laser dinâmico tornou-se o termo usado para denominar o fenômeno que passou a ser usado para monitorar a atividade biológica e não biológica em aplicações desde a agricultura até aquelas relacionadas à medicina. Este fenômeno também é conhecido como biospeckle laser (BSL), quando aplicado em material biológico, e com grande sensibilidade para seguir os menores movimentos intra e intercelulares dos dispersores de luz presentes no tecido biológico. A partir da década de 1970, o biospeckle laser teve sua utilização baseada em uma espiral crescente e ligada ao desenvolvimento tecnológico que favoreceu novas aplicações acompanhadas de novas metodologias de análise. Portanto, a adoção do fenômeno como um sensor, por exemplo, na agricultura e na medicina fomenta sempre uma constante oferta de novos dispositivos e novos métodos favorecendo o uso em novas áreas do conhecimento. Esta revisão apresenta uma resumida história do biospeckle laser (BSL) pontuando as aplicações e desenvolvimentos além dos desafios, como é o caso do uso de forma portátil e por equipamentos comerciais. E esta história foi resumida em um diagrama identificando os chamados pontos do corte com contribuições que mudaram a forma de uso da técnica.

Termos para indexação: Metrologia óptica; biossistemas; desafios.

INTRODUCTION

Speckle pattern is an interference figure, useful in many interferometric techniques, though the presence of grains demands further elimination processing, for example, after obtaining fringes.

By 1975 speckle pattern started to be useful as information to monitor small changes in illuminated objects (Briers, 1975), and it was named dynamic laser speckle or biospeckle laser (BSL) (Aizu; Asajura, 1991) when applied to biological material.

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Applications in Biosystems (Rabal; Braga, 2008) became well known in the literature from medicine (Aizu; Asakura, 1991; Fujii et al., 1987) to agriculture (Oulamara; Tribillon; Duvernoy, 1989; Zdunek et al., 2014), measuring many sample attributes under observation. Thus, the complexity of biological tissues or cells represents great challenge to identify or isolate the major phenomenon responsible for the boiling effect in speckle changes (Cardoso et al., 2011; Braga et al., 2007).

After decades of development and applications, we can identify that the state-of-the art and challenges involving biospeckle usage are mostly circumscribed in research laboratories.

This review presents the history of biospeckle phenomenon as a tool to monitor biological activity in many areas of knowledge, highlighting breakpoints in the field and focusing on the engineering point of view.

HOW CAN WE GET THE BIOSPECKLE INDEX?

The main idea of using biospeckle phenomenon to monitor biological activity is the creation of an index, which indirectly can be match with biological/chemical/physical movements of scatterers in illuminated samples, represented by the biospeckle index (Rivera; Braga, 2017).

Basic arrangements of laser and optics to get the biospeckle signal can be classified in two setups: forward and backscattering. Forward scattering configuration is only possible when sample is transparent and laser can pass through it, carrying information to the camera (Figure 1a). However, this setup presents lower sensitivity than backscattering configuration (Rabal; Braga, 2008), which can be applied to both transparent and opaque samples.

After choosing configuration, the next step is adjusting signal quality. The best option is using an online procedure such as the Motion History Image (MHI) (Godinho et al., 2012), which can be found for free download (http://www.nongnu.org/bsltl/). In Figure 2, it is possible to see that quality test of speckle pattern is followed by data acquisition and processing. If you do not want to use an online procedure to carry out quality test, you can do it offline (Moreira; Cardoso; Braga, 2014). Tests for light intensity, contrast of grains and homogeneity of sample illumination are required, which can be associated with the best f-number selection (Briers; Webster, 1996).

When you have a reliable data, image processing can be done by means of online or offline procedures.

Online processing was addressed by LASCA (Briers; Webster, 1996), which uses only one image to

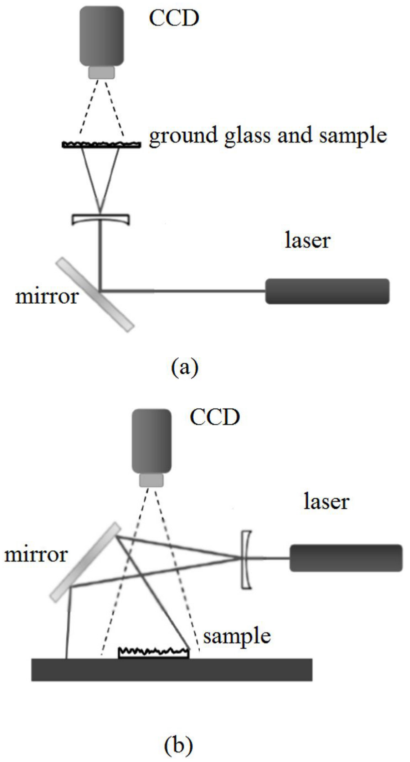

Figure 1: Traditional arrangements of biospeckle laser with (a) forward scattering and with (b) backscattering configuration.

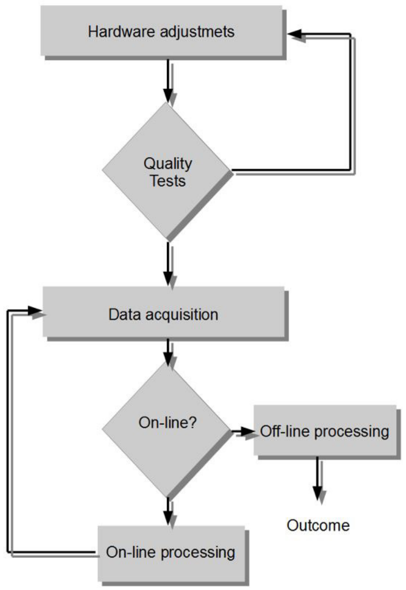

Figure 2: Flowchart representing a biospeckle laser assay with experimental configuration test and image acquisition and processing.

get sample activity by evaluating blurring level in grains, represented by the index contrast (C) (Equation 1).

$$C = 1 - \frac{\sigma I}{< I >} \quad (1)$$

Where σI is the standard deviation of an image portion, and <I> is the mean value of the same portion.

Analysing the image in portions reduces resolution of the outcoming image. Another drawback of LASCA is the need for adjusting the camera and shot speeds to follow changes in the speckle boiling effect (Yuan et al., 2005). This adjustment must be adapted regarding activity change in the illuminated material. Despite the great usage of LASCA in medicine, there are some proposals to overcome limitations, using more than one image to create the online perception (Godinho et al., 2012; Li et al., 2006) without loss of resolution and continuous adjustments.

Offline analysis of BSL can be divided in two fields, the time and frequency domains, with the biospeckle index varying in graphical and numerical approaches. The breakpoint in numerical approaches can be attributed to the creation of a time history represented by a line of points during sample illumination (Oulamara; Tribillon; Duvernoy, 1989). Time history of a line of points was defined as Space-Time Speckle (STS) (Xu; Joenathan; Khorana, 1995) and as Time History of Speckle Pattern (THSP) (Arizaga; Trivi; Rabal, 1999). Multiple indexes are obtained by THSP, and many reports can be found in the literature. An alternative to THSP matrix, formed by a line, is the analysis of the whole image in time, such as presenting the correlation approach (Kurenda; Adamiak; Zdunek, 2012). The high resolution provided by new digital cameras can compromise analysis by time consumption, thus adopting random points around the desired region of interest can be a feasible alternative. (Braga et al., 2017).

In turn, the Fujii method (Fujii et al., 1987) can be considered as the breakpoint in graphical approaches, with improvements proposed by the Generalized Differences (GD) (Arizaga et al., 2002), Standard Deviation (SD) (Nothdurft; Yao, 2005; Blotta et al., 2011), Temporal Difference (Marti-Lopez et al., 2010) and the Parametrized form of Temporal Difference (PTD) (Minz; Nirala, 2014a,b).

Finally, BSL indexes evolution was enhanced when time domain analysis was complemented by frequency analysis (Passoni et al., 2005), which can also be considered a breakpoint. Signal analysis in frequency domain allowed for isolation of desirable bands that could be match with a particular biological phenomenon.

ANALYSIS OF BIOSPECKLE LASER APPLICATIONS

Major areas where biospeckle laser (BSL) usually appears as a non-destructive measurement tool of complex phenomena are medicine and agriculture, with additional applications in food industry as well.

The main advantage of BSL, in most cases, is the ability to measure biological activity by means of indirect indexes overcoming time consumption, human judgement and destructive chemical and/or physical tests defined as Gold Standards.

In medicine, the first applications of dynamic laser speckle occurred back in 1981 (Fercher; Briers, 1981). Many others followed, mostly used for blood flow (Aizu; Asakura, 1991; Briers; Webster, 1996; Fujii et al., 1987). The main characteristic of blood flow measurement is the adoption of online approach to avoid microtremors in live animals and human beings. In the same way, measurement of eye tremor (Kenny; Coakley; Boyle, 2013) was application unrelated to blood flow. Potential applications of LASCA, or contrast method in medicine are enormous. I will not list all of them here, since the objective is to present breakpoints regarding BSL developments rather than provide a list of research works. Otherwise, we can cite some applications in medicine using offline techniques such as identification of cancer adjacent to normal tissue in animal samples (Braga et al., 2012) or even changes in muscle tissues (Maksymenko; Muravsky; Berezyuk, 2015). Offline methods can also be applied to identify parasites (Pomarico et al., 2004; Grassi et al., 2016) or even the effect of drugs in cancer cells (González-Peña et al., 2014).

In agriculture, the breakpoint can be attributed to applications of BSL in fruits (tomatoes), 1975 (Briers, 1975), followed by many other applications in fruits related to maturation stages (Xu; Joenathan; Khorana, 1995; Rabelo et al., 2005; Ansari; Nirala, 2014; Ansari; Nirala, 2016; Nassif et al., 2014; Skic et al., 2016; Retheesh et al., 2016; Costa et al., 2017), or even using fruit to test processes and improve the BSL technique (Oulamara; Tribillon; Duvernoy, 1989; Kurenda et al., 2013; Ansari; Nirala, 2013; Minz; Nirala, 2014a; Minz; Nirala, 2014b; Mulone et al., 2014; Kurenda et al., 2014; Minz; Nirala, 2016; Arefi et al., 2016).

Applications in agriculture and food areas are numerous, and some additional examples include measurement of bull sperm motility, analysis of seeds (Braga et al, 2003; Vivas et al., 2017), root growth monitoring (Braga et al., 2009; Ribeiro et al., 2014), analysis of meat (Amaral et al., 2013; Qingli et al., 2016) and many others. This broad number of applications in agriculture and food areas is an example of potential use and demand of BSL as a sensor.

STATE-OF-THE-ART AND CHALLENGES

Hardware is the real revolution in this area, with, for example, new devices to acquire images. First applications adopted analogue devices whereas today we have a large variety of digital devices using CMOS or CCD sensors embedded in different cameras, from webcams to mini-microscopes, using robust communication protocols such as USB.

The laser adopted was usually the HeNe, 632nm, which was later substituted by solid-state lasers despite doubt about their stability (Ahmed; Yamada; Abdulrhmann, 2001; Ahmed, 2003). Solid-state lasers are smaller than HeNe devices, giving flexibility to build portable equipment including cameras available in small sizes.

Finally, regarding image/signal processing, the development of many routines using a large variety of statistic and mathematical functions was reported in the literature, aiming to address all sort of analysis improvements (Braga; Rivera; Moreira, 2016).

Therefore, I can assume the main challenge to use biospeckle laser systems is their migration to the field or even usage out of optical laboratories. Regarding the software, the profusion of functions presented in the literature poses the main challenge of creating a standard routine, or a group of standard routines, to treat images and provide reliable results. An effort to create a standard is the project Biospeckle Laser Toll Library (BSLTL) (http://www.nongnu.org/bsltl/), where many routines developed during years by several researchers were implemented in M-Code (Matlab and Octave). The library is displayed for free download, associated with raw data, executable software to acquire and play images online, supported by an online tutorial book, also available for free (Braga; Rivera; Moreira, 2016).

An additional effort to classify some routines was conducted regarding spectral approaches, particularly evaluating the hidden filtering process provided (Rivera; Braga, 2017). It was proved that some routines filter images in time, which can compromise the analysis. Thus, the user should be aware of the ideal routine to be adopted in the application desired.

The same behaviour of numerical routines under frequency point of view is presented using graphical outcomes in Figure 3, where it is possible to see the differences in activity maps in three known methods, the Mean values (Braga; Rivera; Moreira, 2016), Fujii (Fujii et al., 1987), Standard Deviation (SD) (Nothdurft; Yao, 2005; Blotta et al., 2011) and the one applied to a well-known raw data related to maize seed (Braga, 2015).

Filtering action is based on the expectation of signal x_n in each point of the image matrix in time, where n represents

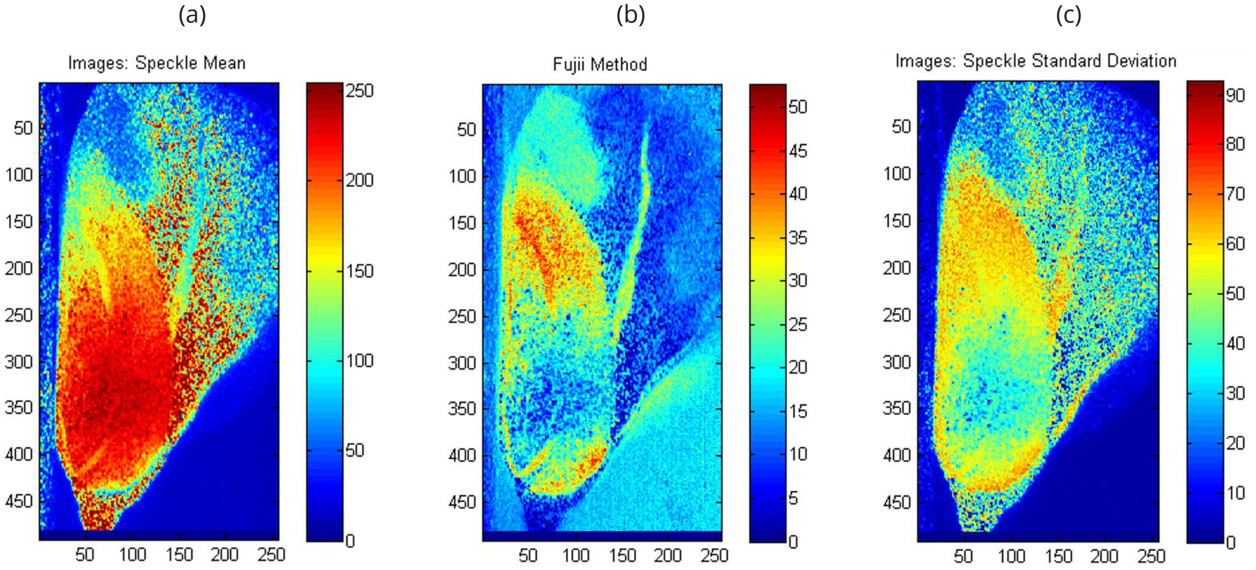

Figure 3: Graphical outcomes of Biospeckle Laser data from maize seed using the routines (a) Mean Values, (b) Fujii and (c) Standard Deviation.

the digital number of values (M) in time. Additional operations presented by Fujii and SD methods also provide cumulative signal filtrations, which can be observed by the user before image processing. In Figure 4, it is possible to see the three operations represented by the aforementioned methods.

Further classification must be done in graphical outcomes to guide users in choosing the best routine to process BSL images.

In Figure 5, we can observe a resume of the main breakpoint in BSL area with additional questions about challenges, or next breakpoints.

Adoption of BSL technology out of optical laboratories will be driven by the challenge to build commercial equipment or even by self-built systems using small, robust and accessible devices such as digital cameras and portable lasers.

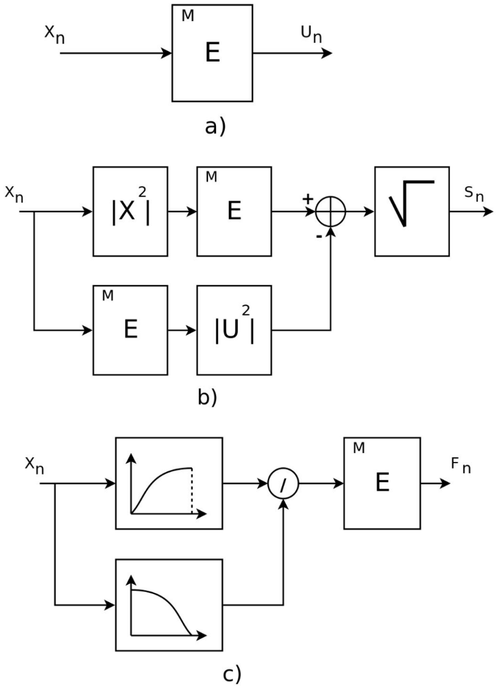

Figure 4: Flow of signal x_n operations representing methods (a) Mean Values, (b) Fujii and (c) Standard Deviation, with E meaning the expectation and U_n, F_n and V_n meaning the respective outcomes, where *n* represents the digital number of values (M) in time.

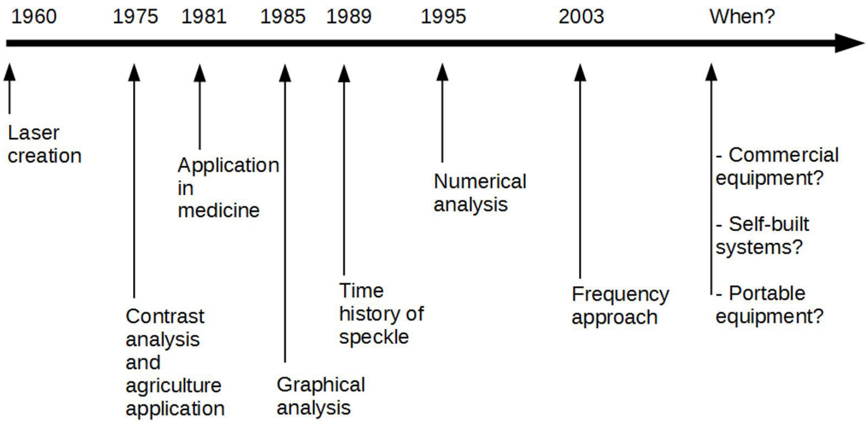

Figure 5: Time line of breakpoints in biospeckle laser development.

Finally, the proposal of BSL as an alternative protocol for biological application can represent a decisive push in the technique.

CONCLUSIONS

Dynamic laser speckle images became a source of information about a broad band of biological phenomena and a tool to monitor sample activities in areas from agriculture to medicine. This tool was named biospeckle laser (BSL) technique, and provides many biospeckle indexes that can be matched to the phenomenon under monitoring. As a sensitive optical tool, BSL is still restricted to optical laboratories but has potential for use also in commercial equipment or even in a portable way. The idea to make it accessible can be based on open source routines associated with self-built systems, using accessible and robust lasers and cameras.

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