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Biospeckle Activity Evolution of Strawberries

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Abstract:
In this work, we measured the biospeckle activity of strawberries (*fragaria* × *ananassa*) with two recently developed algorithms. We observed an important diminution of speckle activity within time periods of 72 h. We tested the methods by analyzing different specimens to check for proper detection of bruised areas or fungal development. The obtained results serve as a starting point to correlate the time evolution of biospeckle activity with aging of fruits, in order to set the basis for development of future standardized fruit quality control procedures.

Keywords:
Biospeckle; Dynamic speckle; Fragaria × Ananassa; Fruit Aging; Mobility Index

1. INTRODUCTION

Visual or photographic images of objects illuminated by an expanded laser beam are covered with a grainy structure that is known as *speckle pattern* or *speckle field*. A speckle pattern consists of light spots with variable intensities and sizes, randomly distributed along the illuminated area. The observed intensity distribution presents no obvious relation to the properties of the surface being illuminated but strongly depends upon the viewing system [1]. The causes originating speckle patterns were recognized to lie in the coherence of the laser light and in the roughness of any surface not ideally specular. As understanding of this phenomenon was developed, several new measurement techniques evolved and were grouped under the denomination of *speckle methods*. Speckle patterns are known to possess information about the illuminated object at a scale below that of the wavelength used (usually called interferometric precision) [2], and that is what speckle methods take advantage of. A speckle pattern is termed dynamic or static, respectively, whether it changes spontaneously or not in a reasonably small time scale (say equal or less than 1 s). A dynamic pattern is usually seen when illuminating biological samples, and thus called *biospeckle*, while static patterns are generally associated with inorganic samples. In the latter case, speckle interferometry methods [3, 4] serve to study small deformations or displacements by comparing the patterns of the sample at two different states, before and after introducing an external perturbation. On the contrary, biospeckle methods are based on studying the spontaneous speckle pattern time evolution of the sample under study [5]. The term biospeckle activity (BA) is generally used to determine the degree

of mobility of the speckle pattern.

The surfaces of most materials (organic or inorganic) are rough on the scale of an optical wavelength ($\sim$ 600 nm). Therefore, in the particular case of organic samples, when laser light with a wavelength above 600 nm is used to illuminate them, the BA does not only depend on its surface but also on its inner tissues. This is due to the penetration depth of light near the bottom edge of the infrared range in biological tissue. As an example of this, the penetration depth of light with a wavelength above 600 nm in apple peel is higher than 2 mm [6] . There are several methods to analyze the temporal evolution of dynamic speckle patterns [7–9]. Pajuelo *et al.* have applied dynamic speckle methods to analyze bruising in apples [10]. Transient processes of melting ice cream have been evaluated also by means of dynamic speckle methods by da Silva Jr. *et al.* [11]. In turn, Kurenda *et al.* investigated temperature effects on apples for fruit quality assessment [12] and recently Amaral *et al.* have used dynamic speckle methods also to evaluate and predict beef quality [13]. However, a clear understanding, a consistent explanation and a standardized method to quantify the BA of fruits and correlate it to fruit quality are still lacking.

In this work we present results obtained from the BA analysis of strawberries, which are amongst the most consumed fruits throughout the world (information extracted from Food and Agriculture Organization of the United Nations, http://www.fao.org) and suffer from rapid post-harvest maturation and aging processes [14]. Therefore, assessment of their quality and estimated shelf-life turns relevant, while, to our knowledge, there is no optical study performed by means of dynamic speckle methods on strawberries. Through the application of two recently developed algorithms for biospeckle analysis [15] we have inspected the correlation between the BA value and the aging or quality characteristics of the fruit, while maintained at room temperature conditions.

2. EXPERIMENTAL DETAILS

2.1 Image Acquisition Setup

A 30 mW diode laser (λ=650 nm) was used as a coherent illumination source and the laser beam was expanded by simply removing the front collimating lens. The fruit was directly illuminated by the expanded beam without diffusers, as can be observed in the simplified scheme of **Figure 1**. A CMOS camera with a 1megapixel resolution was used to record the image sequence of the subjective dynamic speckle patterns formed on the surface of the fruit.

The image acquisition procedure involved three main parameters [15], namely: (i) the number of frames per observation, N ($\geq$ 2), used to calculate the BA value at a given instant, (ii) the time period between those N frames, τ, whose lowest value is limited by the highest temporal resolution attainable by the camera, being generally much greater than the dynamic speckle correlation time (typically $\leq 10^{-4}$ s) [16], and (iii) the time period, t, between subsequent calculations of BA values.

2.2 Speckle Processing Algorithms

For the digital processing of the biospeckle patterns we used two simple algorithms developed in the MATLAB® interface, whose details can be found in [15]. Both algorithms yield a single BA value for the desired region of the sample under analysis. This fact becomes advantageous, since the vast majority of biospeckle evaluation methods provide a matrix of BA values instead (see, for example, [17] and [18]). We proceed now with a brief description of them.

The first method is based on the generalized differences (GD) [19] and Fujii [20] methods. It consists

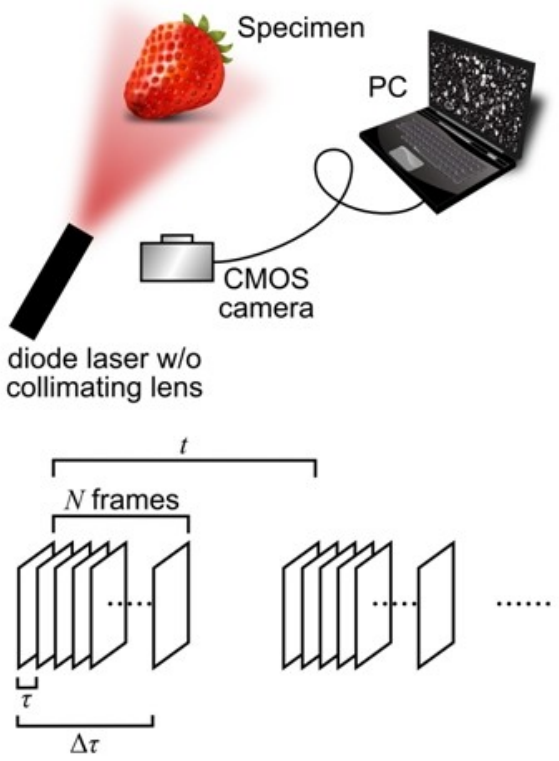

Figure 1. Simplified scheme of the optical setup and the main parameters involved during image acquisition. The diode laser beam is expanded by removing its collimating lens. Each image sequence involves the acquisition of N frames, with a time interval τ between them. From this sequence, a single BA value is calculated for a time instant of length $\delta\tau = (N-1)\tau$. This is repeated after a time t, to measure the time evolution of BA.

on averaging differences between pixel intensities of subsequent speckle images. Therefore, differing from GD or Fujii, this method allows to easily get a single BA value index and quantify the overall degree of mobility of the dynamic speckle pattern at a given time instant of length $\Delta\tau = (N-1)\tau$ (see **Figure 1**). Mathematically, the algorithm can be expressed as

$$\mathrm{BA}_1 = \frac{1}{m \times n} \sum_{k=1}^{N-1} \frac{1}{N-1} \sum_{i=1}^{m} \sum_{j=1}^{n} |I_{k+1}(i,j) - I_k(i,j)|, \tag{1}$$

where BA_1 stands for the BA value obtained from the first method, m and n are the number of rows and columns of each image, respectively, $I_k(i,j)$ is the (i,j) component of the k-th image and N is the number of frames in the sequence. Hence, the BA value calculated in this way measures the image-to-image area averaged absolute pixel intensity variation.

In turn, the second method quantifies the BA by measuring the average fraction of pixels whose intensities change from image to image in more than a certain amount, which we call *speckle noise*. If a speckle pattern is ideally static, i.e. if there is no mobility at all, this fraction would be obviously zero. In the real case, each speckle pattern, whether static or dynamic, is most probably affected by some degree of noise coming from different sources (vibrations, ambient dust, electronics, thermal fluctuations, and so on). The amount of noise of the acquisition system may be estimated in several ways by measuring the variation of pixel intensity in a speckle pattern which *a priori* should be static. We have estimated the noise level, r, by means of taking the maximum pixel to pixel absolute intensity variation in a region R of the N images where the speckle pattern is supposed to be static. Mathematically this is expressed as

$$r = \max_{k=1\ldots N-1} \left\{ \left| R(i,j)_{k+1}^{static} - R(i,j)_{k}^{static} \right| \right\}, \tag{2}$$

where k covers all possible values from 1 to $N-1$ and now i and j sweep over all pixels of region R. The *static* superscript remarks the fact that this calculation has to be performed on a region R of the N images where the speckle pattern must be nearly static. Once the noise level is approximated, the algorithm proceeds with the following operation

$$\mathrm{BA}_2 = \frac{1}{m \times n} \sum_{k=1}^{N-1} \frac{1}{N-1} \sum_{i=1}^{m} \sum_{j=1}^{n} \Theta \left[|I_{k+1}(i,j) - I_k(i,j)| - r \right], \tag{3}$$

where now the BA_2 value is the result obtained from the second method, the scaling factor $(m \times n)^{-1}$ transforms the obtained quantity to the desired area fraction of varying pixels, $\Theta[x]$ is the well-known Heaviside function and, obviously, by multiplying this quantity by 100 we get a percentagevalue.

2.3 Analyzed Samples

We analyzed four different strawberries (*fragaria* × *ananassa*) which will be referred as *A*, *B*, *C* and *D* from now on. Samples *B*, *C* and *D* were taken from the same stock to ensure they have the same age, while sample *A* was extracted from a totally different stock. Strawberries have a quick ripening process, as mentioned before, for which we expected to detect significant BA variations during a few days.

Sample *A* was analyzed by calculating first its BA value at $t = 0$ h and then at $t = 24$ h. Each time, a sequence of $N = 10$ photographs was acquired with a time interval $\tau = 1$ s between them (see **Figure 1**). For the rest of the samples, the BA calculation was performed every ($t =$) 30 min, taking also $N = 10$ photographs separated by a $\tau = 1$ s time interval. In this way, sample *B* was observed during 87 h, sample *C* during 92 h and sample *D* during 74 h. Sample *D* was frozen at –12 °C for 24 h previous to the experiment, in order to hasten the ripening process.

3. RESULTS AND DISCUSSION

Sample *A* was used as a test for the algorithms and allowed us to prove that the BA values diminished appreciably from one day to the next by making only two observations, separated by a 24 h period. The BA_1 value dropped from 3.0 to 1.9, while the BA_2 value decayed from 65% to 28%. Relative errors were around 10% in both cases. In **Figure 2** we present the image representations of sample *A*, obtained by means of extracting data from the first algorithm, at the (a) first and (b) second day of observation.

These images clearly show that as cellular activity decayed from one day to the next, the image definition deteriorated at some regions of the strawberry. This could be observed easily with the naked eye and was further confirmed by calculating the pixel intensity mean value, which underwent almost a 40% diminution between both states. The BA_1 values obtained for sample *B* ranged between 10.0 and 7.2, with relative errors below 6%. In turn, we obtained BA_2 values starting at 84.4% and decreasing to 70.5% with also a maximum relative error of 6%.

In **Figure 3** we present the BA curves obtained from both methods, showing the decreasing trend for sample *B* as a function of time. There is a slight fluctuation above and below the average trend curves, but the monotonous overall diminution is clear. The curves have a similar shape, which evidences that both algorithms are consistent with each other. It is worth noting that these values are quite high in relation to those of sample *A*, but we recall here that this sample was brought from a different strawberry stock.

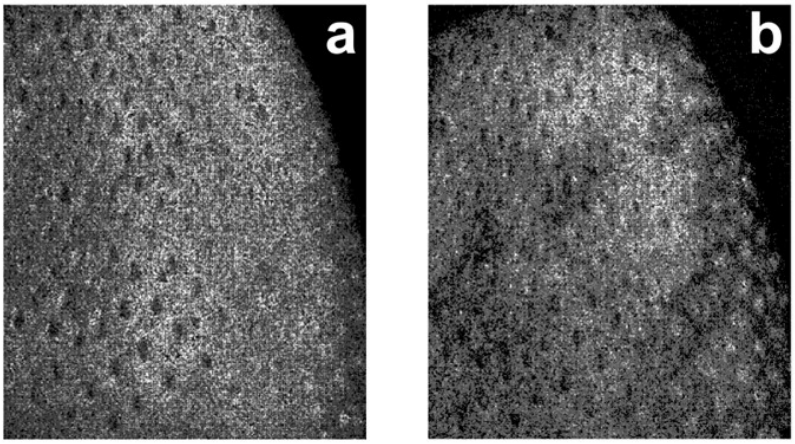

Figure 2. Images of sample *A* reconstructed from the first algorithm at (a) the first day and (b) the second day. The appearance of black zones in (b) denotes BA diminution.

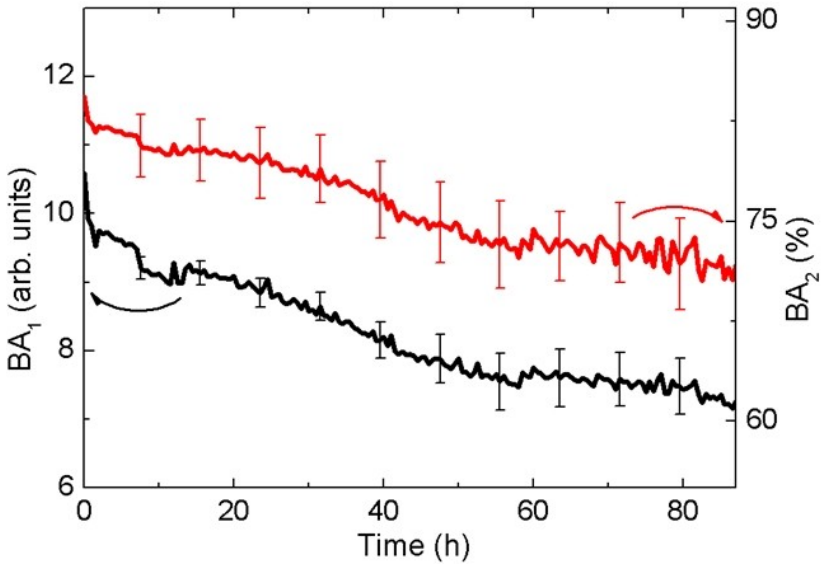

Figure 3. Evolution of BA values for sample *B* as a function of time, obtained by both methods. The curves present nearly the same monotonous diminution trend during 87 h of observation.

The BA_1 calculations for sample *C* yielded values in the range between 5.0 and 1.2, while BA_2 values ranged between 87% and 43%. Relative errors were quite high (reaching 60%) for this strawberry when calculating BA_1 values. In turn, relative errors for BA_2 were much lower and around 10%. The evolution of BA_1 and BA_2 values through the 92 h observation period is shown in **Figure 4**. This sample was characterized by the existence of a black region in its surface, which was intentionally put facing the camera to analyze the influence on the obtained BA values of surface defects on the fruit. This region corresponded, in this particular case, to a bruised area. Therefore, this explains the fact that the BA values for sample *C* were lower than those obtained for sample *B*. We also observed for sample *C* a monotonous BA diminution during approximately 60 h, followed by a slight increment for longer times. This unexpected behavior conducted us to analyze the cause for that, for which we just continued observing the fruit.

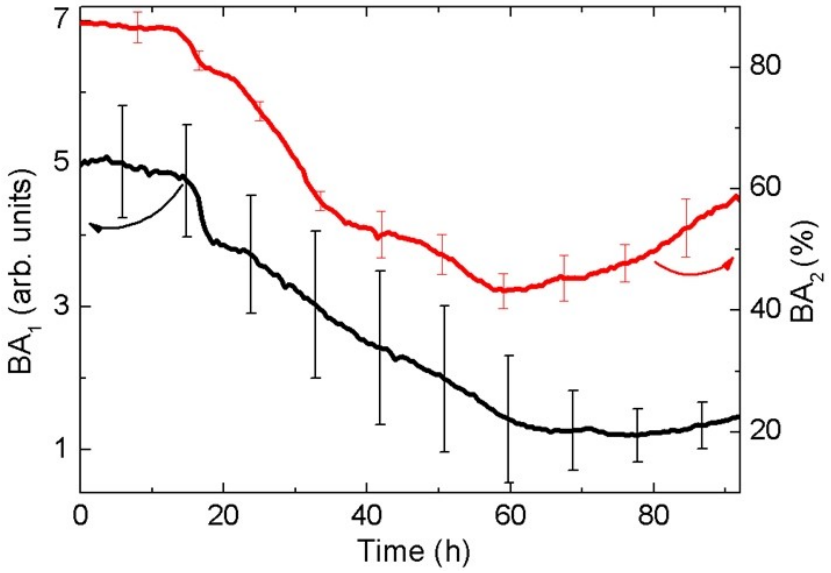

Figure 4. Evolution of BA values for sample *C* as a function of time, obtained by both methods. The curves presented a reversal of the decaying trend, which was due to growth of fungi colonies as was visually confirmed after continued observation of the fruit.

Remarkably, we noticed the appearance of fungi in the initially bruised region to which we attributed the reversal of the BA diminishing trend. The detection of fungi by means of dynamic speckle methods has already been reported by other authors [17].

Sample *D*, which was previously frozen, yielded BA_1 values ranging between 9.0 and 0.6 and BA_2 values decreasing from 81% to 10%. Here again, relative errors for BA_1 reached values as high as 65% and remained below 20% for BA_2. The BA evolution curves are shown in **Figure 5**. Both curves presented an important diminution during the first 5 h period and continued to decrease subsequently at a lower rate. After 40 h of observation, the diminution rate augmented until BA reached the lowest values throughout all samples with both algorithms. This is coincident with the fact that sample *D* was frozen and, consequently, its cells were damaged.

Results obtained for all samples showed a typical response of ripening biological tissues, i.e. a BA diminution as the fruit ages [5]. The observed fluctuations of the curves are related to the characteristic randomness of biospeckle patterns.

Sample *B* presented the highest BA values, being this coincident between both algorithms. This motivated us to assert that sample *B* should be the highest quality fruit among the studied group of strawberries coming from the same stock, i.e. among samples *B*, *C* and *D*. Our assertion is supported by these evidences: (i) sample *C* has a bruised area, which we intentionally located in the region under analysis and effectively resulted in appreciably lower initial BA values, (ii) sample *D* was frozen prior to analysis, which also resulted in slightly lower initial BA values and a quicker diminution of activity as compared to sample *B*, (iii) sample *D*, which was previously frozen, yielded the lowest BA values after an observation period of around 60 h with respect to samples *B* and *C* and (iv) relative errors resulting from BA calculations for sample *B* were lower than those for both samples *C* and *D*. Relative errors resulted systematically higher for BA_1 calculations as compared to BA_2. The reason for this is that the averaging procedure in (1) takes into account the intensity of all pixels without considering if they changed or not. However, the calculation procedure for BA_2 given in (3) involves an averaging procedure only among those pixels whose intensity varies above a certain threshold value, given by (2). Such high initial BA

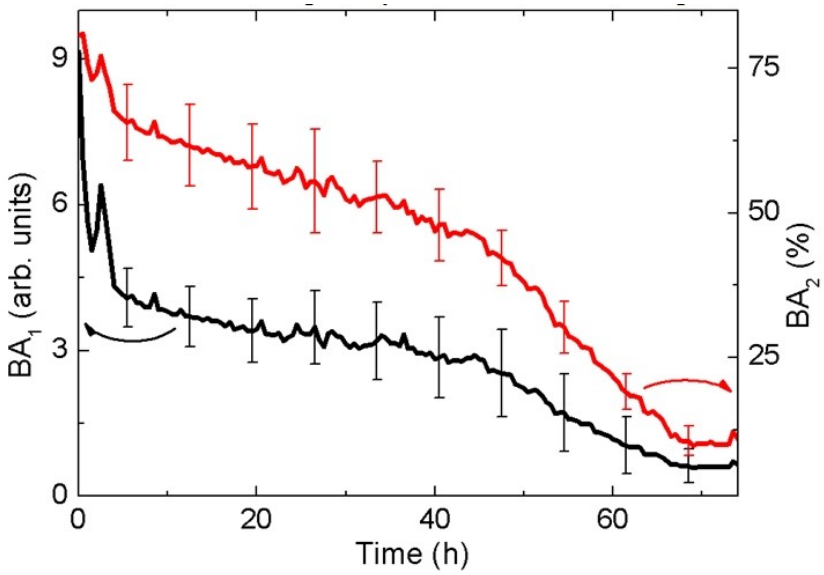

Figure 5. Evolution of BA values for sample *D* (previously frozen) as a function of time, obtained by both methods. The curves presented a higher diminution rate as compared to the other samples. Abrupt decay during the first 5 h was attributed to the defrosting process of the fruit.

values of sample *D* are attributed to the defrosting process of the strawberry (i.e. humidity) rather than to the biological activity of the fruit. Sample *C* also served us, unexpectedly, to infer that fungi colonies could be detected by these methods provided a sufficiently long observation period. In this particular case, a time period of around 24 h was necessary to detect the reversal of the decaying trend in the BA curve (see **Figure 4**). We are currently working on fungi detection by means of dynamic speckle methods [21], however this issue exceeds the aim of the present article and will be further analyzed in future works.

4. CONCLUSION

We have inspected the evolution of the BA of strawberries by means of applying two simple algorithms that were proposed recently [15]. The results obtained from both methods were comparable for all samples and showed a decaying biological activity as a function of time, as was expected. The viability to identify different conservation states of a strawberry has been demonstrated. Both methods allowed distinguishing between good quality fruits (i.e. recently harvested or well preserved) from one that is at an advanced stage of maturation. We have also checked that BA values differ between a healthy area and a damaged or bruised one, which allow inspecting the state of different zones of the same fruit. Moreover, these methods allowed for an early detection of fungi colonies growing onto the surface of the fruit. In conclusion, the results presented in this work allowed us to assert that the employed evaluation methods appear as useful tools for fruit quality assessment. Further research efforts will be pointed towards this issue.

5. ACKNOWLEDGEMENTS

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References

[1] T. Kreis, "Holographic Interferometry," in *Handbook of Holographic Interferometry: Optical and Digital Methods*, pp. 35–219, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, 2005.

[2] R. Jones and C. Wykes, *Holographic and Speckle Interferometry (Cambridge Studies in Modern Optics). (2nd Edition)*, vol. 6. Cambridge university press, 1989.

[3] P. Jacquot, "Speckle Interferometry: A Review of the Principal Methods in Use for Experimental Mechanics Applications," *Strain*, vol. 44, no. 1, pp. 57–69, 2008.

[4] I. T. Nistea and D. N. Borza, "High speed speckle interferometry for experimental analysis of dynamic phenomena," *Optics and Lasers in Engineering*, vol. 51, no. 4, pp. 453–459, 2013.

[5] H. J. Rabal and R. A. Braga Jr, *Dynamic Laser Speckle and Applications*. CRC Press Taylor & Francis Group, Boca Raton Fl. 181-220, 2009.

[6] J. Lammertyn, A. Peirs, J. De Baerdemaeker, and B. Nicolaı, "Light penetration properties of NIR radiation in fruit with respect to non-destructive quality assessment," *Postharvest Biology and Technology*, vol. 18, no. 2, pp. 121–132, 2000.

[7] A. Federico and G. H. Kaufmann, "Evaluation of dynamic speckle activity using the empirical mode decomposition method," *Optics communications*, vol. 267, no. 2, pp. 287–294, 2006.

[8] M. F. Limia, A. M. Nuñez, H. Rabal, and M. Trivi, "Wavelet transform analysis of dynamic speckle patterns texture," *Applied optics*, vol. 41, no. 32, pp. 6745–6750, 2002.

[9] J. D. Briers and S. Webster, "Laser speckle contrast analysis: a nonscanning, full-field technique for monitoring capillary blood flow," *Journal of biomedical optics*, vol. 1, no. 2, pp. 174–179, 1996.

[10] M. Pajuelo, G. Baldwin, H. Rabal, N. Cap, R. Arizaga, and M. Trivi, "Bio-speckle assessment of bruising in fruits," *Optics and Lasers in Engineering*, vol. 40, no. 1, pp. 13–24, 2003.

[11] E. Da Silva Jr, E. R. T. Da Silva, M. Muramatsu, and S. C. d. S. Lannes, "Transient process in ice creams evaluated by laser speckles," *Food Research International*, vol. 43, no. 5, pp. 1470–1475, 2010.

[12] A. Kurenda, A. Adamiak, and A. Zdunek, "Temperature effect on apple biospeckle activity evaluated with different indices," *Postharvest Biology and Technology*, vol. 67, pp. 118–123, 2012.

[13] I. C. Amaral, R. A. Braga Jr, E. M. Ramos, A. L. S. Ramos, and E. A. R. Roxael, "Application of biospeckle laser technique for determining biological phenomena related to beef aging," *Journal of Food Engineering*, vol. 119, no. 1, pp. 135–139, 2013.

[14] M. Holzwarth, S. Korhummel, R. Carle, and D. R. Kammerer, "Evaluation of the effects of different freezing and thawing methods on color, polyphenol and ascorbic acid retention in strawberries (fragaria × ananassa duch.)," *Food Research International*, vol. 48, no. 1, pp. 241–248, 2012.

[15] N. Budini, C. Mulone, F. Vincitorio, C. Freyre, A. López, and A. Ramil, "Two simple methods for overall determination of mobility in dynamic speckle patterns," *Optik-International Journal for Light and Electron Optics*, vol. 124, no. 24, pp. 6565–6569, 2013.

[16] P. Zakharov, A. Völker, M. Wyss, F. Haiss, N. Calcinaghi, C. Zunzunegui, A. Buck, F. Scheffold, and B. Weber, "Dynamic laser speckle imaging of cerebral blood flow," *Optics express*, vol. 17, no. 16, pp. 13904–13917, 2009.

[17] R. A. Braga Jr, G. F. Rabelo, L. R. Granato, E. F. Santos, J. C. Machado, R. Arizaga, H. J. Rabal, and M. Trivi, "Detection of fungi in beans by the laser biospeckle technique," *Biosystems engineering*, vol. 91, no. 4, pp. 465–469, 2005.

[18] A. Zdunek, A. Adamiak, P. M. Pieczywek, and A. Kurenda, "The biospeckle method for the investigation of agricultural crops: A review," *Optics and Lasers in Engineering*, vol. 52, pp. 276–285, 2014.

[19] R. Arizaga, N. Lucı, M. Trivi, *et al.*, "Display of local activity using dynamical speckle patterns," *Optical Engineering*, vol. 41, no. 2, pp. 287–294, 2002.

[20] H. Fujii, K. Nohira, Y. Yamamoto, H. Ikawa, and T. Ohura, "Turbidity and Microbial Risk in Drinking Water," *Applied Optics*, vol. 26, no. 24, pp. 5321–5325, 1987.

[21] F. Vincitorio, N. Budini, C. Mulone, M. Spector, C. Freyre, A. L. Díaz, and A. Ramil, "Detection of fungi colony growth on bones by dynamic speckle," in *8th Ibero American Optics Meeting/11th Latin American Meeting on Optics, Lasers, and Applications*, pp. 8785D2–8785D2, International Society for Optics and Photonics, 2013.

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