Assessment of bio-speckle activity of lemon fruit

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Abstract: Bio-speckle can be used as a method for analysing the activity of biological materials illuminated with laser beam. Physically, bio-speckles are the result of scattering of coherent light on moving particles inside living tissue. Therefore, the aim of this study was to investigate bio-speckle activity in lemons and determine the age of the lemon fruit from the observation of its dynamic speckle pattern. The speckle pattern of laser light scattered in lemon fruits were measured through their quantification. For the quantification of the variation by bio-speckle, two different methods of image analysis were used: moment of inertia and the spatial temporal speckle correlation coefficient. The bio-speckle activities were also characterized by the line profiles of time history speckle pattern (THSP) which contains data of time information of dynamic speckle. Furthermore, measure of the dynamic speckle varies for fruits as their quality decrease, and the values change with the position where the images are taken.

Keywords: bio-speckle, Time history speckle Pattern, line profile, lemon, activity.

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1 Introduction

Lemon (*Citrus limon*) is economically important fruit in the world, which is grown in developed and developing countries and certainly constitutes one of the main sources of vitamin C. Lemon have firm, smooth skins, juicy flesh and few seeds. The sour taste of lemons is caused by the presence of organic acids. The major acid in lemons is citric acid, which constitutes around 5% to 6% of lemon's juice. Further, they contain other vitamins such as vitamin B, riboflavin and minerals like calcium, phosphorous and magnesium besides proteins and carbohydrates. Lemons are known to reduce the risk of heart diseases, cancer and work as antiseptic, astringent, digestive stimulant etc.

There is an increasing demand of "high quality fresh citrus" driven by World Health Organization recommendations. Thus, fruits have been a matter of extensive research in recent years because of their importance to agriculture and human diet. The knowledge of fruit maturity is important for harvesting and post-harvesting process (Rabelo, 2005). The literature presents many techniques developed to evaluate the maturity of fruits as well as of any plant organ, based on their mechanical behaviour that have been employed to study the mechanical resistance of vegetative tissues in general. These mechanical tests are supported by the classical elastic and viscoelastic laws and principles including failure theories, assuming the basic conditions required by the mechanics of continuous media. However, the continuity, isotropicity and homogeneity of vegetative materials by no means matches the characteristics of the ideal material, which generates an uncertainty associated to the real mechanical behaviour of the tested samples. The history of the mechanical evaluation of vegetative tissues includes simple compression, stress and strain rates controlled tests, impact test, acoustic tests and lately, optical material behaviour based tests. It should be emphasized that the above-referred uncertainty required new tests to serve as means of comparison and checking of the encountered mechanical properties (Rabelo, 2005).

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Mechanical tests are widely used in agricultural practice to determine, quality or date of harvesting because of its simplicity and low price. However, during last twenty years a number of optical (including laser-based) methods were developed which equally well as mechanical tests are used in agriculture. Excellent reviews in this topic are available such as quality measurement of fruits and vegetables (Abbott, 1999) and non-destructive measurement of fruit and vegetable quality (Nicolaï et al. 2014). Recent use of biospeckle to evaluate the quality of agricultural products has been reported by Zdunek et al. (2014).

The research evolution indicates strong interests in developing tests based upon machine vision and laser instrumentation qualified as non-destructive measuring systems (Rabelo, 2005). Therefore this paper reports an alternative for non-destructive tests based on laser techniques, which has been studied and pointed out as a potential application in shelf life prediction of fruits and vegetables. The laser technique consists in studying the temporal variations of the dynamic speckle on lemons. The dynamic speckle phenomenon occurs when laser light is scattered by bodies exhibiting some activity, as the biological material does. The visual appearance of such a phenomenon is similar to a surface of a boiling liquid, originating the denomination of dynamical speckle, boiling speckle or biospeckle. Recent researches based on the identification of the speckle pattern for different biological materials indicate the application of the dynamical speckle in correlation with mechanical properties, seed viability, staining, drying. Despite the complexity of the phenomenon, some approaches have been developed to quantify the temporal variation of the speckle pattern to characterize biological changes (Rabelo, 2005). In order to quantify temporal variations of the biospeckle, two approaches are employed: (a) the first one, based on the calculation of the moment of inertia of the co-occurrence matrix of the temporal history of speckle pattern (THSP) and (b) the second one, spatial temporal speckle correlation coefficient. The THSP was proposed by Xu et al. (1995). The advantage of using biospeckle characterization, in the study of biological material, relies on the fact that the proposed method is a fast and non-invasive measurement procedure. According to Rabelo (2000), the complexity of the involved phenomena does not permit a simple and reliable approach of a model involving the interaction of light and biological material.

The next biological material is very complex and the distribution of its constituents is not uniform within the whole sample. Therefore, we can expect that the biospeckle will be different over the illuminated surface, with the differences in color and scars being additional factors to be considered. In order to show these differences, some experiments with lemons are reported in this paper.

2 Materials and methods

2.1 Dynamic speckle

The parameters of a speckle pattern and its behaviour strongly depend on the numerical aperture and diameter of the illuminating beam, the wave front curvature at the object plane, and observer location. Speckle motion can be categorized as boiling and translation motion. In boiling motion, the speckles change their shape and size with the motion of the object. Translation speckle motion is related to the motion of the speckle pattern as a whole. In pure boiling motion there is no point in defining speckle displacement. Usually in arbitrary conditions there is no pure boiling or translation motion of speckles, but a mixture of both. The translation/boiling regime of speckle motion is defined as a ratio of the translation distance, r_T, to the average speckle radius, r_S (Yoshimura, 1986):

$$\eta = \frac{r_T}{r_S} \quad (1)$$

Where,

$$r_T = \left(1 + \frac{D_S}{R_W}\right).r_B \quad (2)$$

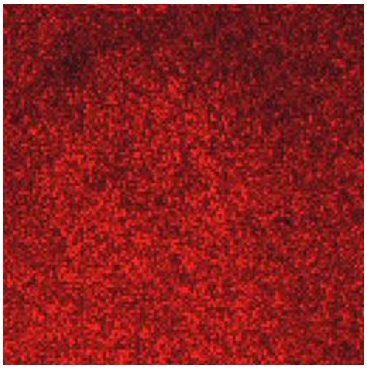
$$r_S = \frac{D_S.\lambda}{\pi.r_B} \quad (3)$$

Here the translation distance, r_T is calculated for the illumination with a Gaussian beam (Yoshimura, 1986);

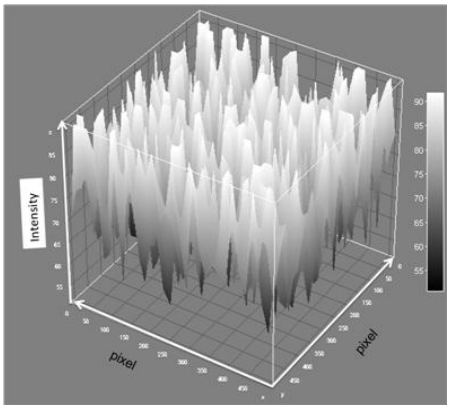
D_S is the distance between the illumination and observation point; R_W is the illuminating beam wave-front curvature at the object plane; r_B is the radius of the spot on the object; λ is a wavelength generated with a laser.

According to Equation 1, if η = 0, the dynamic speckles show pure boiling; if | η | < 1, boiling becomes dominant; if | η | > 1, translation becomes dominant. Using this factor η, one can evaluate two types of motion of dynamic speckles.

Figure 1 shows a typical speckle and its intensity distribution scattered from the lemon fruit surface upon laser illumination for very short duration.



a.



b.

Figure 1 (a) A typical speckle scattered from lemon surface, (b) 3D intensity pattern of the speckle

2.2 Inertia moment

Arizaga et al. (1999) developed a process based on the occurrence of successive intensity values in the whole THSP image. The process requires the transformation of the THSP in an occurrence matrix, called co-occurrence matrix (COM), defined by Equation 4.

$$COM = \left[N_{ij} \right] \quad (4)$$

The entries N_{ij} of the co-occurrence matrix are the number of occurrences of a certain intensity value i followed by an intensity value j, which according to Arizaga et al. (1999) characterizes a particular case of the spatial gray level dependence matrix, employed to characterize the image texture. Figure 2 presents the construction of THSP and its corresponding co-occurrence matrix.

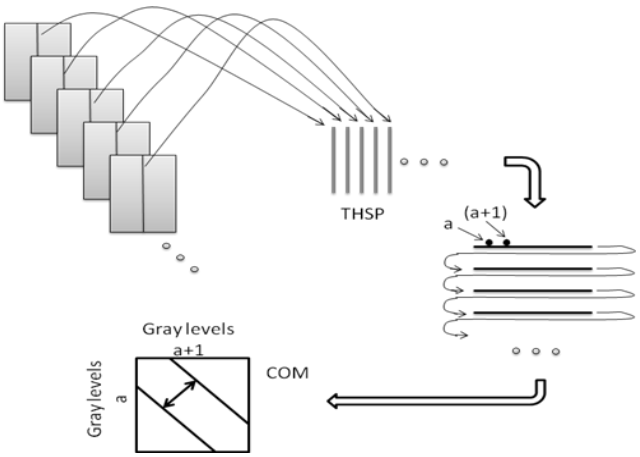
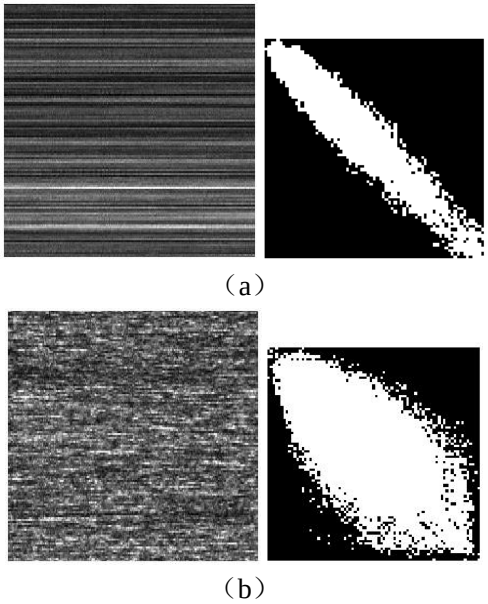


Figure 2 Construction of temporal history speckle pattern (THSP) matrix and its co-occurrence matrix

Figure 3a shows the THSP of a low activity sample and its corresponding modified co-occurrence matrix (MCOM). Figure 3b shows the THSP of a high activity sample together with its corresponding co-occurrence matrix.

The differences between the two samples mentioned above can be noted by comparing either the THSP or the MCOM showed in Figures 3a and 3b. The nonzero values out of the main diagonal indicate changes in the intensity of two successive pixels. The dispersion of the points in the matrix indicates the activity level of the biological sample. If the values are concentrated around the main diagonal in the MCOM, the pixels in the THSP do not change their intensity value, which means that the sample presents a low activity.



（a）

（b）

Figure 3(a) THSP and MCOM of low and (b) high activity sample

The differences between the Figures above have to be quantified, as a measure of the biological activity. An alternative way to characterize speckle time evolution is based on the co-occurrence matrix (Haberacker, 1985; Zobrist, 1975 and Kruger, 1974) of the intensity in the time domain.

A measurement of the spread of the matrix values around the principal diagonal with these features can be constructed as the sum of the matrix values times its squared row distance to the principal diagonal. This is a particular second order moment called the inertia moment (IM) of the matrix, with respect to its principal diagonal in the row direction, as presented in Equation 5.

$$IM = \sum_{ij} M_{ij}(i-j)^2 \qquad (5)$$

Where，

$$M_{ij} = \frac{N_{ij}}{\sum_{j} N_{ij}} \qquad (6)$$

is the normalization of co-occurrence matrix (COM) called modified co-occurrence matrix (MCOM). This measurement (IM) is a useful tool to estimate the global activity in the several biological and non-biological applications with a summary value (Rabal, 2009).

The occurrences in the diagonal do not contribute to increasing the IM value, while far way M entries add their more heavily weighted values.

2.3 Spatial-Temporal speckle cross-correlation analysis

This technique is based on the correlation analysis of two or more speckle patterns, where one is considered as an image of a reference state. The reference pattern and the patterns of subsequent object states are divided into an equal number of regions and then cross-correlation of each pair of respective fragments is calculated. The calculation of the cross-correlation coefficients for a series of speckle pattern sub-images recorded in the given temporal order allows the temporal dependencies of these coefficients to be received as functions of the biospeckle pattern movement speed (Zdunek, 2007). Each such dependency is equivalent to temporal degradation of a correlation peak.

For analysis biospeckle activity (BA) was evaluated using the correlation coefficient $C^{k\tau}$, where k is frame number and τ is lag time (1/0.7 s) (Zdunek, 2007). $C^{k\tau}$ was calculated as the correlation coefficient of data matrix, consisting of intensities of pixels, of the first frame with the data matrixes of the following frames from the analysed biospeckle movies. In this study, C was analysed only as the correlation coefficient between the first frame and the frames at kτ = 0.4 s and 0.7 s. Then, a BA = 1 − $C^{0.4}$ value was determined as the biospeckle activity parameter for the sample. Higher biospeckle activity corresponds to higher 1 − $C^{0.4}$ value. Correlation coefficient C was calculated using “corrcoef” function in Matlab® R2010a software (MathWorks, USA).

For the first frame $k\tau = 0$ s and $C^{k\tau} = 1$. When the speckle pattern shows no temporal fluctuation over a duration of τ s, i.e. k = 0 = constant and $C^{k\tau} = 1$ as shown in Figure 4. However, when speckle pattern changes in time, the value of $C^{k\tau}$ decreases fastly below the constant value 1 as shown in the Figure 4. Thus, biospeckle activity, BA can be defined as:

BA = Value of C when speckle shows no temporal change

– value of C when speckle shows temporal change over τ s

$$BA = 1 - C^{k\tau} \quad (7)$$

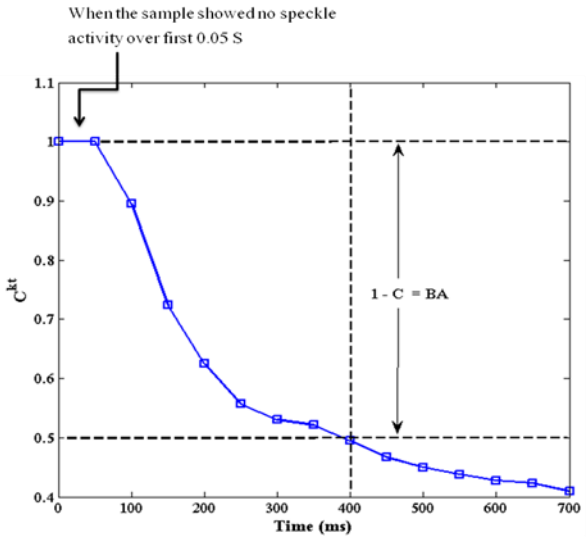


Figure 4 Calculation of biospeckle activity (BA)

2.4 Experimental procedure

Tests were carried out using lemon fruits taken from local growers at mature yellow stage. The experimental procedure is shown in the figure 5. A 10 mW helium-neon laser beam with a neutral density filter was used to illuminate the samples and a CCD (Basler-scA1300-32fc- of resolution 1294×964 pixels with frame rate 32 fps and pixel size 3.75×3.75) camera with objective as the recording device. Biospeckle laser analysis was done at room temperature, in the absence of light, movements or noise inside the laboratory. Lemon samples were illuminated by the laser light for 12.75 seconds (s) needed to get 256 images under a time rate of 0.05 s.

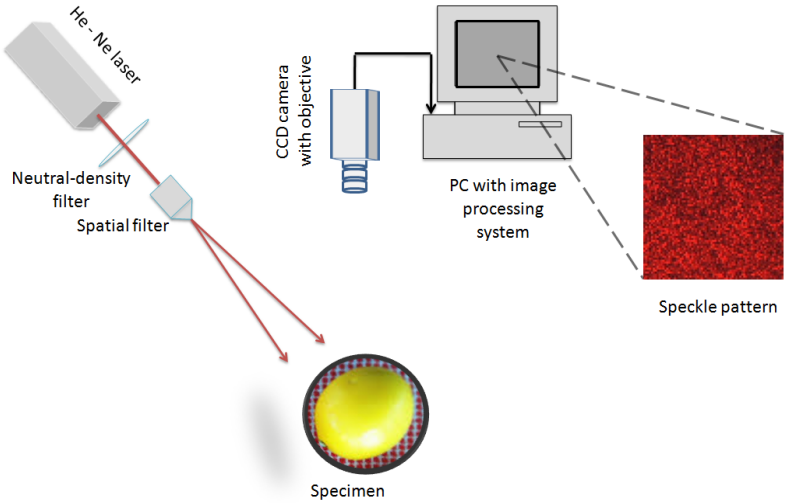


Figure 5 Experimental configuration of lemon biospeckle laser

A column of speckle pattern image was recorded every 0.05 seconds and a composite image of 256×256 pixel size was generated by storing consecutive columns digitized to eight bit gray levels. Average laser illumination was kept constant during the measurements by using a neutral density filter for all samples. Possible influences of the specular reflections of the sample were not taken into account as they don't significantly affect THSP formation (Rabelo, 2005). The storage period was the most important cause of quality loses in lemons, which were qualified through the biospeckle interpretation. Fifteen lemons were selected with similar size and mass. Information about the biospeckle time variations was obtained, allowing calculating the moment of inertia of the co-occurrence matrix of the THSP and the biospeckle activity (BA).

Four points were chosen over the surface of lemons to test their biospeckle activity. Points A and B were located in the equatorial region, point C in the peduncle insertion, and point D placed in the apex region of the

fruit. Figure 6 shows the points over the surface of the lemon (A, B - equatorial region; C - peduncle insertion and D - apex).

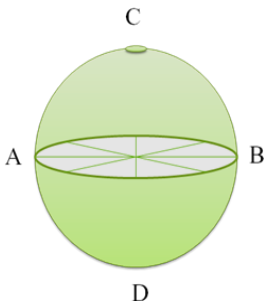
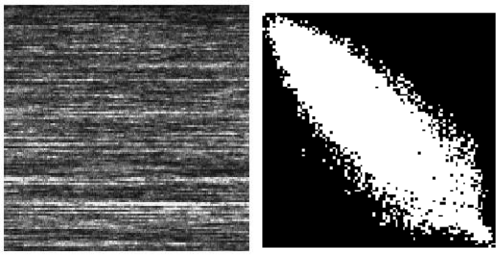


Figure 6 Schematic representation of the positions where images were taken on the surface of lemon: A, B – equator, C – peduncle insertion and D – apex

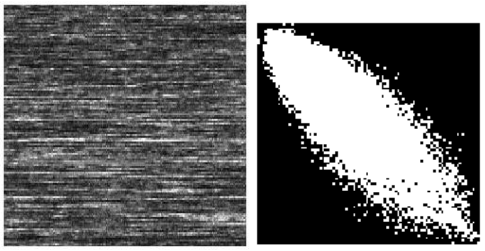
3 Results and discussion

3.1 Experimental results

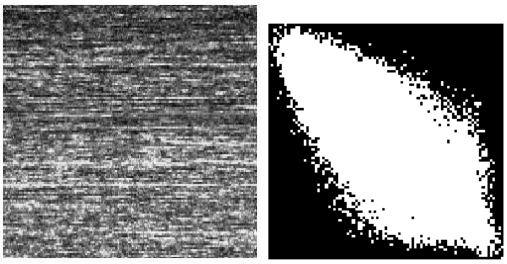
The results of THSP carried out on lemons are represented in Figure 7, for one fruit, and each diagram is constructed with 256 vertical pixels representing the spatial speckle and 256 horizontal pixels representing the temporal variation of the speckle. These diagrams show that the fruit exhibits a different THSP pattern, depending on the illuminated point, which can be observed through the continuity of the lines on it. Similar results were obtained for all the fruits. These diagrams do not permit a precise evaluation through a direct observation. It is necessary to generate numerical values associated to them. These values can be expressed by the moment of inertia, which would be able to distinguish the differences among the fruits. The moment of inertia and the biospeckle activity (BA) associated to the images for the four different points on the fruit were calculated. Tables 1 presents the average values of the moment of inertia.



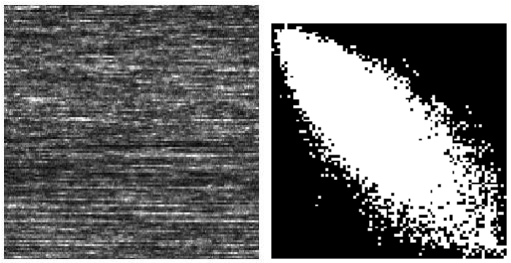
(a)



(b)



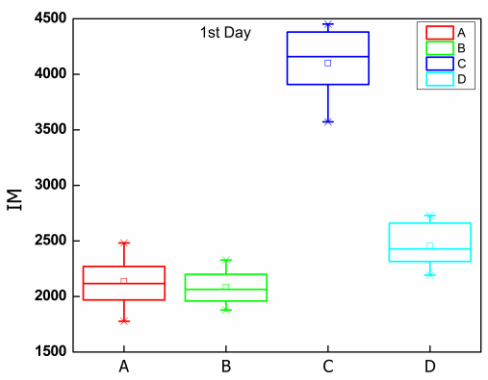
(c)

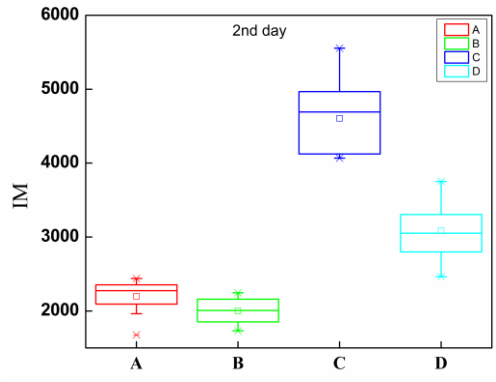


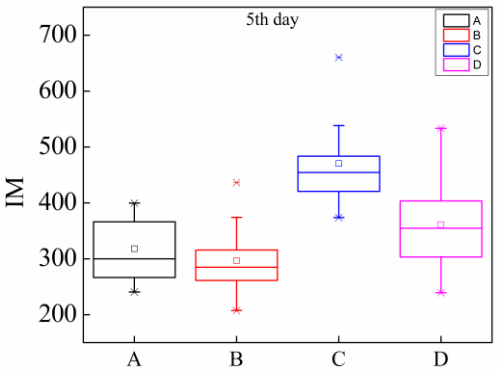
(d)

Figure 7 THSP and MCOM for images taken on positions: (a),(b) – equator, (c) – peduncle insertion and (d) – apex (two days)

Figure 8 shows the daily variation of IM values for one specimen, where it is possible to compare the evolution of the process by IM. IM approach presents the ability to separate fresh highly active and less active fruits.







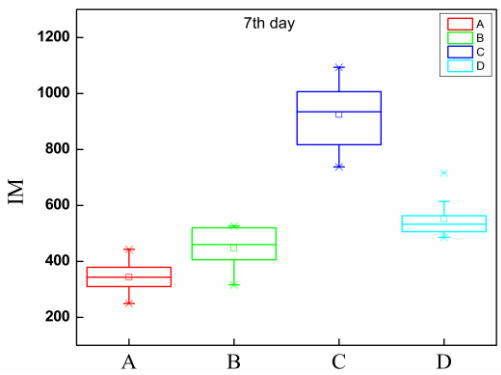


Figure 8 Inertia Moment (IM) values estimated at different points of illuminations (A, B – equator, C – peduncle insertion and D – apex) on lemon surface (during different days of storage)As the lemon ages, IM values decrease as is shown in Table 1. Point C presents high activity where the peduncle is inserted. The IM values for points A and B statistically do not present significant differences. Therefore, the points C and D are the points that allow identifying the activity differences between the maturation stages of the fruit.

Table 1 Average values of inertia moments

Days of storage	Inertia moment values	Point of illumination of the lemon fruit			
		A	B	C	D
1	Mean*	2138.41	2085.67	4101.97	2455.41
	SD	196.87	140.40	307.43	176.58
	SE	50.83	36.25	79.38	45.60
2	Mean	2198.00	1998.46	4605.85	3088.89
	SD	205.79	171.41	453.03	324.03
	SE	53.14	44.26	116.97	83.66
5	Mean	317.93	296.52	470.19	360.98
	SD	52.93	58.96	77.11	83.78
	SE	13.67	15.22	19.91	21.63
7	Mean	343.90	447.36	924.76	552.41
	SD	49.50	73.94	115.34	71.59
	SE	12.78	19.09	29.78	18.48

* n=number of measures per sample

Figure 9 shows that how IM values change with the frequency of the temporal speckle pattern consisting of 256 images. On the first two days when the samples were fresh IM value increases rapidly and gives the variation of pixel or intensity value with time, but when the activity of biological sample goes on decreasing with time the IM value decreases. The influence of the higher changes in the activity is represented by the abrupt changes in the THSP pixels.

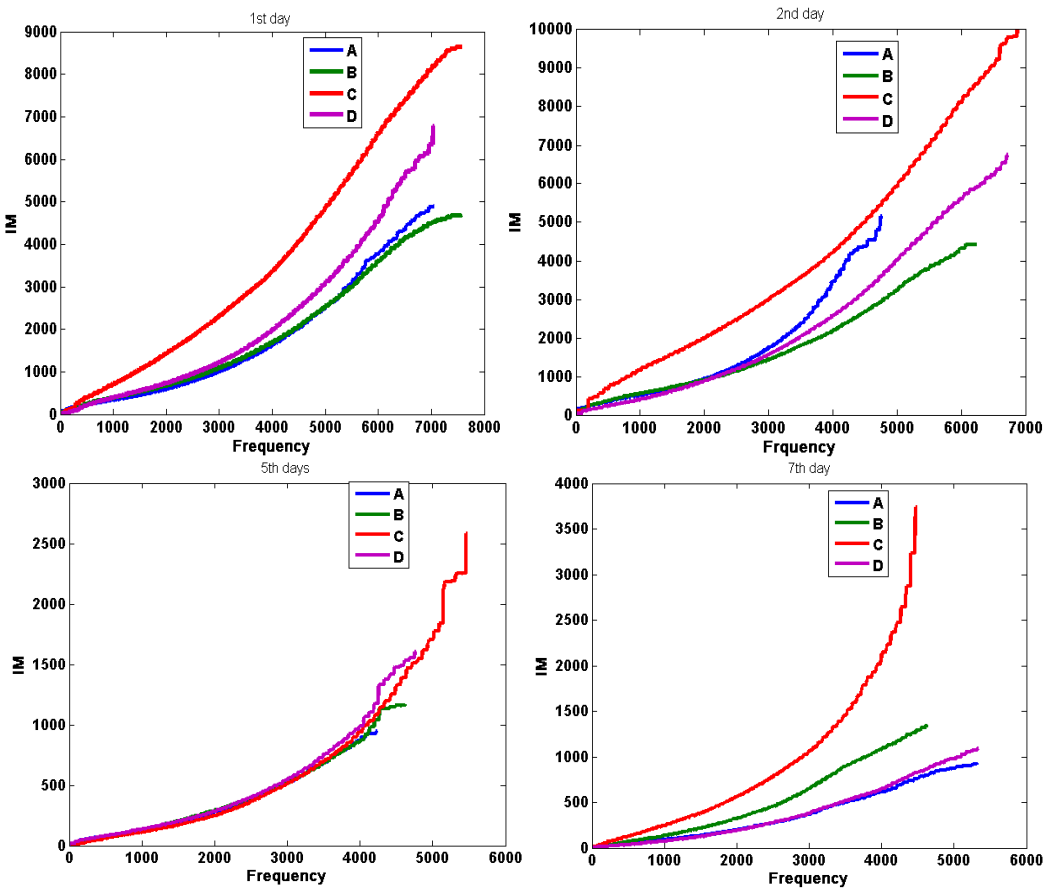


Figure 9 Variation of IM value with the frequency of dynamic intensity of the biospeckle movie composed of 256 frames recorded at every 0.05 second

Figure 10 presents THSP images, which show different patterns of variation in intensities for each point of illumination. Equation 1.5 indicates that the main information in the IM calculation is the difference between two immediate pixels, so, if the time history is characterized by a low frequency, modulating the curve, that low frequency behaviour will not be represented in the IM calculation. So IM results can be directly related to line profiles of the respective THSP images.

Figure 10 shows three different activities characterized by the line profiles of the respective THSP images. The fast variations are responsible to raise the value of IM. It is exemplified with respective IM values representing the major influence of high frequencies in IM values.

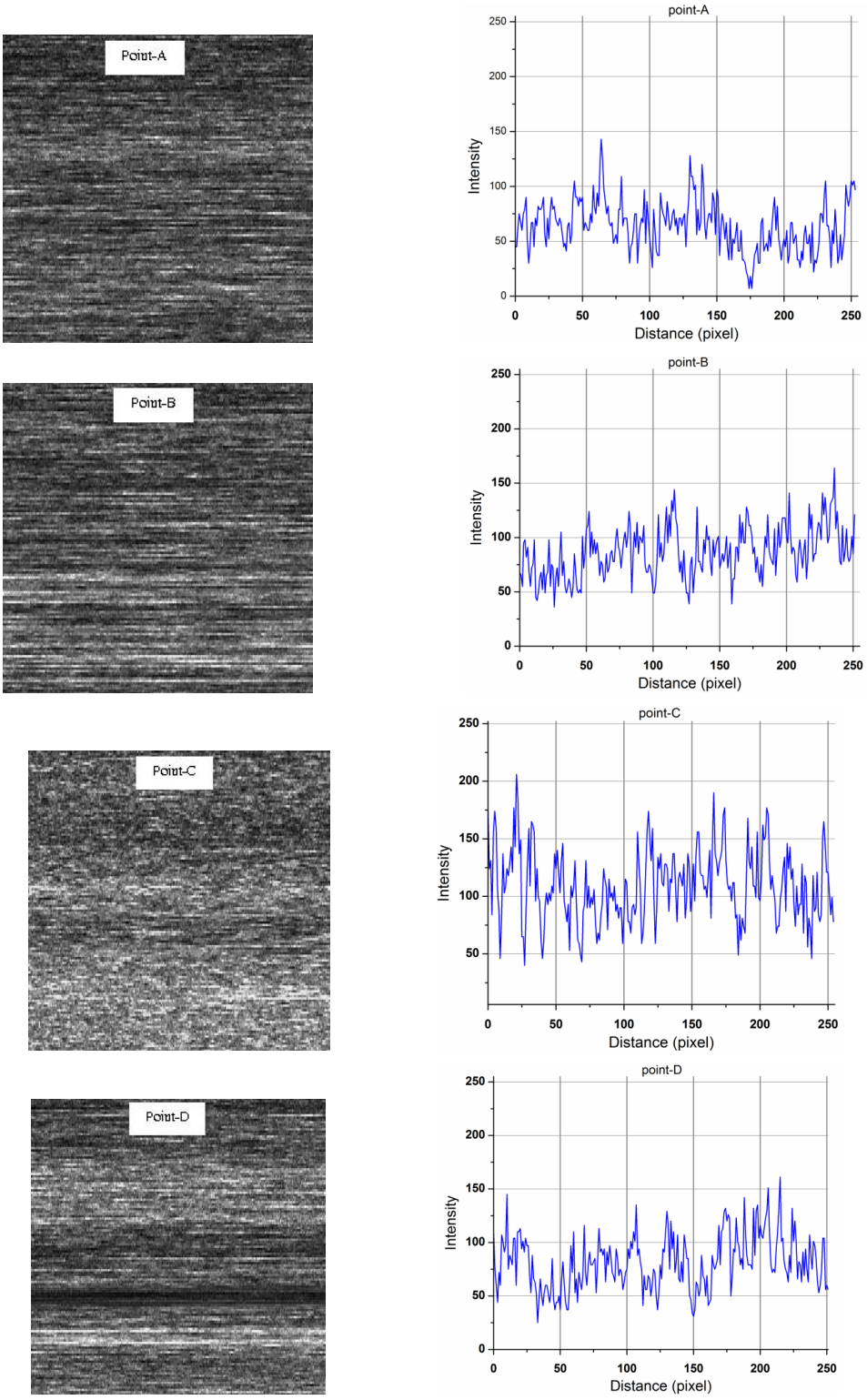


Figure 10 The graphic shows THSP at different points of illumination and respective line profiles (gray level) with different IM values. Note the major influence of high frequencies on IM values: (a) IM = 2198, (b) IM = 1998.46, (c) IM = 4605.85, and (d) IM = 3088.89.

Figure 11 shows temporal changes of cross correlation coefficients $C^{k\tau}$ at points (A, B - equatorial region; C - peduncle insertion and D - apex) at 2nd day of storage of lemon. When the lemons were fresh at 2nd day of storage, the $C^{k\tau}$ value decreased rapidly and the decay is more rapid at the insertion region C. The insertion point C presents relatively high biospeckle activity (BA) as calculated in Table 2 for one specimen.

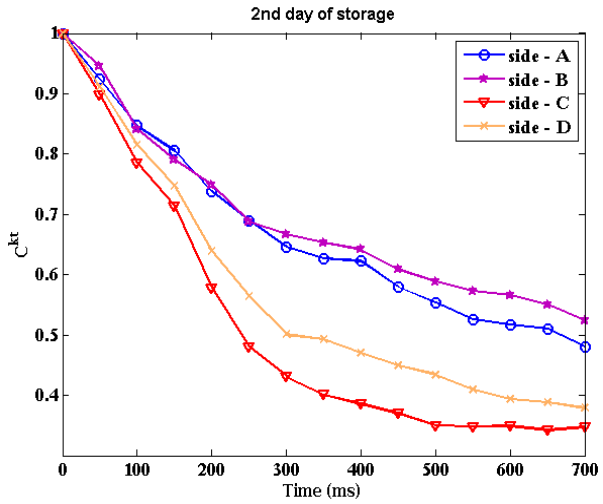


Figure 11 Temporal changes of cross correlation coefficients during storage of lemon

Table 2 Biospeckle activity (BA) calculation at different points of illumination

Point of illumination	BA at 0.4 s	BA at 0.7 s
A	0.3775	0.5190
B	0.3580	0.4762
C	0.6139	0.6526
D	0.5298	0.6208

3.2 Discussion

It has been experimentally verified that biospeckle laser technique is suitable for the analysis of biological processes occurring during the lemon fruit maturation. The measure of the biospeckle activity varies in different stages of post-harvest. It is also possible to get useful information from fruits THSP patterns by comparing how the frequencies, that compose the signal vary in time. Taking into account the practical application of the biospeckle method to assess the biological activity of fruit, the methods, inertia moment and correlation coefficient showed significant results. These results indicate that this non-destructive test might be used successfully in shelf-life prediction of fruit and vegetables. The methods might be used also to yield a new, commercial technique to assess the quality of fruits.

4 Conclusions

1) THSP evolution allows identifying the activity differences between the maturity stages of the fruit.

2) IM approach presents the ability to separate highly active and less active fruits. The influence of the higher changes in the activity is represented by the abrupt changes in the THSP pixels. The fast variations are responsible to raise the value of IM. It is exemplified with respective IM values representing the major influence of high frequencies in IM values.

3) Moment of inertia values and cross correlation coefficients decrease as the post-harvest period of the lemon fruits increases. Thus the measure of the dynamic speckle varies for fruits in different stages.

4) Biospeckle activity can be characterized by the line profile of time history speckle pattern (THSP) which contains data of time information of dynamic speckle. The inertia moment value depends on the frequency of the line profile of time history speckle pattern (THSP). The fast variations of the line profile signal are responsible to raise the value of IM.

5) Measure of the dynamic speckle changes with the point of illumination where the images were taken. Fruits show complex environment and thus dynamic speckle depends on the different points of illumination showing different environment.

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