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Biospeckle activity measurement of Indian fruits using the methods of cross-correlation and inertia moments

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ABSTRACT

This paper presents biospeckle activity evaluation using two methods namely spatial–temporal speckle correlation and inertia moment applied for three different Indian fruits namely apple, pear and tomato for the first time. The bioactivity was determined by means of the cross-correlation functions of the intensity fluctuations and using inertia moment of the THSP image of biospeckles. Significant changes in bioactivity were observed during their shelf lives. From the study, it is found that the activity is higher for pear in comparison to the apple and tomato as predicted by IM method. Biospeckle activity decreases with aging of the fruits but the decrease is more in pear & relatively less in the case of apple and tomato as predicted by cross correlation technique. Further it is also concluded that the activity changes according to their respiration rates. By the comparative study between the two methods it is found that IM is more reliable to predict the bioactivity levels in fruits in comparison to the cross-correlation technique as IM measures the bioactivity directly.

1. Introduction

Due to the socioeconomic importance of guaranteeing quality food, there is a persistent search for the improvement of food products in terms of safety, health, appearance, and many other market attributes. So there is need to evaluate fruits quality at different stages of pre- and post-harvest technology in order to provide product of the best quality to consumers [1]. Recently, a few interesting optical techniques and devices have been developed and successfully used for nondestructive evaluation of fruit and vegetables: vis/NIR spectrophotometry [2], time-resolved reflectance spectroscopy [3], hyperspectral backscattering imaging [4,5], laser-induced light backscattering [6,7] or chlorophyll fluorescence [8–10]. Nikolai et al. [11] have reviewed most of the above techniques, collectively naming them NIR spectroscopy.

Biospeckle is a phenomenon that occurs when laser light reaches an object that exhibits some kind of activity or dynamic process that can be biological or non biological. It is an optical technique for nondestructive evaluation of biological materials. In the method, coherent laser light illuminates an object to be investigated. The backscattered light interferes and a speckle pattern is created in an observation plane. If the sample does not show activity, the speckle pattern is stable in time. However in the case of biological samples, the speckle pattern consists of two components: the static one from stationary elements of the tissue and the variable one from moving particles of the tissue. The variable in time speckle pattern is characteristic for biological tissue and has been called as the biospeckle.

When the speckle is formed by the portion of light scattered by movable elements, it is modulated by their movement. Then, it is difficult to identify precisely which element is responsible for the scattering, because a laser photon can penetrate the vegetal material and suffer multiple deviations in its path before it eventually returns to the surface and reaches the light detector. Cellular structure may vary from one specimen to another, and the movement of cell components can also vary. This movement is also modified by the age of the cell. Therefore, it can be expected that the speckle formed by different cells is different; in addition, the speckle will change as the cell ages. In other words, a speckle can be used to distinguish specimens and also the age of biological material. We consider the age of a fruit as the number of days passed since the sample was brought from the mother plant. Temporal analysis of speckle can be used to estimate the age of fruits after harvest, assuming the correlation function as a sort of parameter to classify differences in speckle.

Bragga et al. [12] have shown that processes related with movement of the scattering centers in the tissue, such as cytoplasmic streaming, organelle movement, cell growth and division during fruits maturation and biochemical reactions are responsible for a

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certain biospeckle activity. Brownian motions should be considered as a source of biospeckle activity too. It has also been shown that biospeckle activity changes with an age or with some surface properties, for example an infection of a biological object. It is also found that biospeckle activity changes with water [13], chlorophyll and starch contents [14,15]. Less chlorophyll content causes higher apparent biospeckle activity [15] due to light absorption by this pigment and in consequence shallower light penetration through a tissue.

So far, attempts to apply biospeckle methods in biological studies include measurements of blood flow in blood vessels [16], viability of seeds [17,18], activity of parasites in living tissues [19,20], analysis of maturation and bruising of fruits and vegetables [21,22]. These studies showed that decaying of a tissue conditions caused by age, illness/infection or damage, relates with lower biospeckle activity.

In this paper two different methods known as spatial–temporal speckle correlation technique and Inertia Moment have been utilized to interpret biospeckle data of three different Indian fruits namely apple, pear and tomato. The methods are noncontact and non-destructive and have been used for the bioactivity evaluation of fruits during their shelf-lives.

2. Biospeckle cross-correlation method

Spatial–temporal speckle correlation (DSC) technique is a measuring process based on the correlation analysis of a reference speckle pattern of the specimen in its initial state with sequential speckle patterns while changing the surface or subsurface of the specimen [23].

In order to obtain the temporal dependencies of biospeckle pattern movement speed, each pattern is separated on M by N sub images and each (m, n)th subimage is correlated with the respective subimage belonging to any other pattern of the same studied area. The cross-correlation function of respective fragment pairs with identical indexing can be expressed as [24]:

$$C_{m,n}(k,l) = \frac{1}{IJ}\sum_{i}^{I}\sum_{j}^{J} r_{m,n}(i,j)s_{m,n}(i+k, j+l) \tag{1}$$

where $r_{m,n}$ is the (m, n)th fragment of the pattern 1, $s_{m,n}$ is the (m, n)th fragment of the pattern 2, $m = 1,\ldots,M$ and $n = 1,\ldots,N$ are the numbers of fragments, $i = 1,\ldots, I$ and $j = 1,\ldots,J$ are the numbers of fragment pixels, $k = 1,\ldots,K$ and $l = 1,\ldots,L$ are the discrete samples of the cross-correlation function.

To obtain the temporal dependencies of biospeckle pattern movement speed, each pattern was separated on M by N sub images and each (m, n)th sub image was correlated with respective sub image belonging to any other pattern of the same studied area. As a result, cross-correlation coefficients were obtained using the equation:

$$C_{m,n}^{k\tau} = \left| \frac{\left\langle \left(S_{i,j}^{t_0} - \left\langle S_{i,j}^{t_0} \right\rangle \right) \left(S_{i,j}^{t_0+k\tau} - \left\langle S_{i,j}^{t_0+k\tau} \right\rangle \right) \right\rangle}{\sigma_{i,j}^{t_0}\sigma_{i,j}^{t_0+k\tau}} \right| \tag{2}$$

where i, j is the pixel number in the (m,n)th sub image of the digital biospeckle pattern, $i = 1,\ldots,I$; $j = 1,\ldots,J$; $m = 1,\ldots,M$; $n = 1,\ldots,N$; $S_{i,j}$ is the i, jth pixel intensity, k is the number of biospeckle patterns, τ is the interval between two adjacent frames containing recorded biospeckle patterns, $\sigma_{i,j} = \sqrt{\left\langle \left(S_{i,j} - \left\langle S_{i,j} \right\rangle \right)^2 \right\rangle}$ is the variance. Calculation of the cross-correlation coefficients for series of speckle pattern's sub images recorded in the given temporal order allows receiving the temporal dependencies of these coefficients as functions of the biospeckle pattern movement speed. Due to homogeneity of biospeckle properties of each surface fragment, intensity of which is calculated as a mean value of intensities of all correlation peaks and the correlation coefficient can be expressed as:

$$C^{k\tau} = \left| \frac{\left\langle \left(S_{im,jn}^{t_0} - \left\langle S_{im,jn}^{t_0} \right\rangle \right) \left(S_{im,jn}^{t_0+k\tau} - \left\langle S_{im,jn}^{t_0+k\tau} \right\rangle \right) \right\rangle}{\sigma_{im,jn}^{t_0}\sigma_{im,jn}^{t_0+k\tau}} \right| \tag{3}$$

where $im = 1,\ldots I,\ldots,2I,\ldots,MI$ and $jn = 1,\ldots J,\ldots,2J,\ldots,NJ$.

Table 1
Bioactivity of different Indian fruits during their shelf lives.

Fruit commodity	Shelf life (days)	BA (average)
Apple	1	0.523
	2	0.476
	4	0.454
	6	0.426
Pear	1	0.617
	2	0.591
	4	0.521
	6	0.478
Tomato	1	0.081
	2	0.076
	4	0.068
	6	0.061

BA, bioactivity.

2.1. Experimental

In order to study the biospeckle temporal properties of the fruits experimental setup was mounted. An expended He–Ne laser (2 mW) with $\lambda = 632.8$ nm, beam was used for recording the biospeckle patterns of the fruits with a CCD camera connected to PC. The optical part of the setup was kept on the vibration free table for decreasing the influence of external perturbations. For the experiment, the recording time was equal to 15 s with the frame rate of 20 fps. The observation area was marked on each of them. Measurements were performed every day on the same places of the fruits.

The images of speckles were obtained as a movie. The movies were then recorded as speckle pattern series (series of images) and they were processed using PC by special code developed for this purpose. The code calculated the correlation coefficients $C^{k\tau}$ according to Eq. (3). These data were used to plot the temporal dependencies of these coefficients.

We planned our study as follows-we selected three types of different climacteric (with starch reserves) Indian fruits namely apple, pear and tomato. The fruits were fresh and conditioned at room temperature for one day before a shelf life program consisting of 1, 2, 3, 4, 5, 6 and 7 days of storage. We have plotted cross correlation coefficients for all the three fruits on inter-day basis. We have taken a series of observations for the fruits and repeated these observations three times almost in identical situation with temperature in the range of 24–27 °C and humidity in the range of 62–65%.

2.2. Results

Biospeckle activity (BA) was calculated using the correlation coefficient $C^{k\tau}$, where $k = 0, 1, 2, 3,\ldots$ and $\tau = 1/15$ s. $C^{k\tau}$ was calculated as the correlation coefficient of data matrix of the first frame ($k = 0$) with the data matrixes of the following frames (at $k\tau$) from the bitmaps of the biospeckle. In this study, ^{14}C was analyzed only as the correlation coefficient between the first frame $k\tau = 0$ and the frame at $k\tau = 14$ s. Then, biospeckle activity BA = 1 − ^{14}C value