Study of biological activity in apple through speckle image processing

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Abstract: This study investigates the ripening phases of apples using speckle image processing. A coherent laser source irradiates the bio-sample, generating a self-interference pattern characterised by bio speckle tiny bright and dark spots. The dynamic speckle pattern captures intensity distribution over time and is analysed through a greyscale co-occurrence matrix, serving as a texture descriptor for bio speckle activity. The correlation between surface roughness and maturation stages is examined, with speckle activity quantified using a bright and dark pixel counting method.

Keywords: Bio speckle; Grey level co-occurrence matrix; image processing; B&D pixels.

I. INTRODUCTION

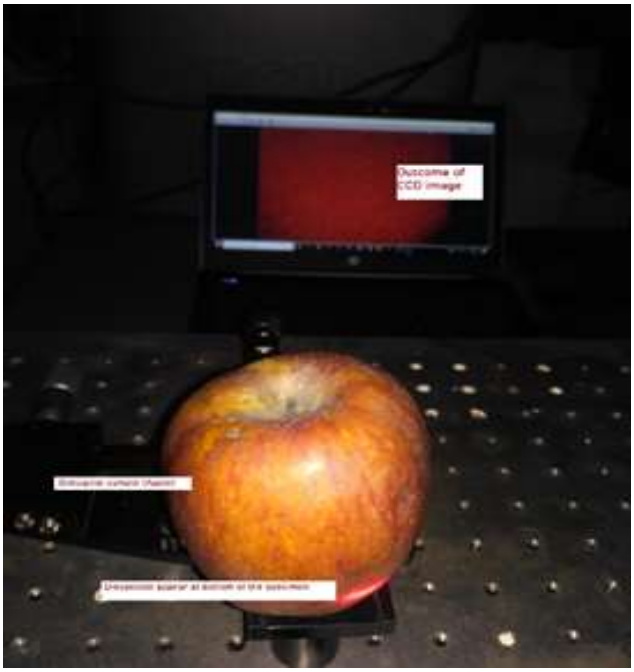
The quality of an apple's post-harvest life is crucial for consistent marketing and serves as an important indicator of ripeness, which significantly influences consumer perception and satisfaction [1]. Apple ripening is related to its softening and physicochemical changes. Vis/NIR spectrophotometry [2], reflectance spectroscopy [3], hyperspectral backscattering imaging [4], chlorophyll fluorescence imaging [5] and laser-induced backscattering [6] are the usual optical methods for the appraisal of the feature of fruits. However, these demonstrations of time-based variants of speckle fluctuation throughout the shelf-life have a limited scope. Bio speckle is a characteristic phenomenon of bio-specimen to evaluate the maturity stages by non-destructive assessment of biological activity. The occurrence of illuminating laser light on a biological specimen and recording the backscattered self-interference tiny spots, known as speckle image pattern. Surface rigidity of the biological sample changes due to the ageing effect and the movement of internal particulates. Hence, the intensity and shape of the speckle image are changeable in time [7]. This time-dependent speckle occurrence is termed as bio speckle, a worthy characteristic tool for the analysis of fruits and vegetables [8]. This is also called boiling speckle because its appearance resembles boiling liquid during the dynamic stages. The Association of intensities of biological specimens brings speckle changes due to the scattering laser light [9]. Temporal de-correlation effect and correlation function of speckle fluctuations are

studied for different fruits by image processing technique [10]. The biological sample experiences chemical composition changes and cellular structure due to the ageing effect and advanced intensity fluctuations or bio speckle activity. Instrumental and manual backup destructive and chemical methods are customarily involved in the features associated with fruit ripening. Boiling speckle sequences are correlated to estimate the maturing stage of fruits and vegetables, by different factors like soluble solids, firmness and acidity through the quantification of biological activity. The Physicochemical deviations that occur inside the cells at that time of the ripening progression of the sample increase the biological activity. The metabolic, enzymatic and respiratory activities are causes for the intense scattering of the laser.

In this work, the investigation of the bio speckle activity of the sample fruit is studied by the Time History of Speckle Pattern (THSP) method. The roughness of the specimen surface analysis and bright and dark pixel methods are described the bio speckle activity. The mathematical analysis of THSP with Co-occurrence Matrix (COM) delivers the intensity distribution of speckle with respect to time.

II.EXPERIMENTAL SETUP

A coherence of He-Ne laser of 2 mW power and 632.8nm wavelength with beam expander and spatial filter is used. The laser beam falls on the specimen suitably as depicted in Figure 1. The scattered laser from the surface of the sample creates a speckle pattern in space. The 30˚angle is suitable to diminish the undesirable specular reflection. The dynamic activity of bio speckle depends on the very mild drive of organelles and particles on a cellular scale. Pixel resolution 800 X 600 has been chosen to obtain a suitable observation area of about 2x2 mm of the specimen pericarp. The optical recording system is equipped with an f-number of 20fps with a recording time of 10 seconds. A small aperture is preferred to observe the bio speckle activity. The laser source is kept at a distance of about 200 mm away from the specimen in the optical system. The sequences of observations are made on the sample in a similar condition with a temperature range of 21–26°C and humidity range of 55–60% with a small, focused spot about 1mm on the surface of the sample neglect the influence of the surface curvature) and the positions recorded are illustrated in Figure 1&2.



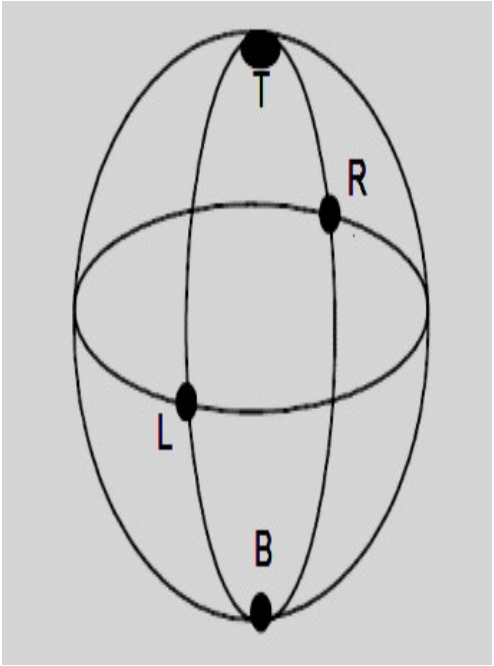


Figure 1. Bio speckle on the biological sample

Figure 2. Schematic representation of the positions: L, R – equator, B –peduncle insertion and T – apex.

III.RESULTS AND DISCUSSION

The study of the mobility of scatters in biological objects is realized by second-order temporal statistics. To obtain 2D information, we use a high-resolution CCD camera for recording. The intensity of the speckle image is documented by a CCD camera with 20 frames per second. 512 consequent speckle images are captured and a fixed column particularly, the central column has been grabbed. With the help of stack speckle images by 512 columns, a single speckle image formed with 512x 512 pixels and digitized to 8 bits 256 grayscale for storage. The resultant speckle pattern is identified as Space-Time Speckle (STS) in which each row embodies the time progression of laser greatness. Totally 256 options are there for characterising the pixel values from 0 (Dark) to 255 (Bright) highpoints to the study of B&D pixel counting.

3.1. Computation of speckle pattern using GLCM

Statistical method, model-based analysis, geometrical oriented, and signal processing are used to sample surface texture study. GLCM is a statistical measurement technique to describe the connection among the neighbouring groups of pixels in the image. It indicates how often pairs of grey-level pixels are disjointed by a fixed distance 'd' and lie beside in the way 'θ' of a speckle image.

The GLCM compromises the joint probability density to express the distribution of pixels with the same grey value. The number of times repeating grayscales to a specific direction and offset can be determined by this method. This normalized value is the COM of the cell. Energy, contrast, homogeneity and correlation are the important features obtained by the GLCM [12]. Contrast estimates the confined variations in the grey-level COM; energy evaluates the uniformity; homogeneity specifies the nearness of the distribution of elements in the GLCM diagonal. Entropy measures the disorder of an image. Speckle image correlation shows the linearity of greyscale with the surrounding pixels. The positively correlated image represented as 1 and the negative correlation is denoted as -1.

$$\text{Contrast} = \sum_{i,j=0}^{N-1} P_{i,j}(i-j)^2 \quad \text{....(1)}$$

$$\text{Energy} = \sum_{i,j=0}^{N-1} P_{i,j}^2 \quad \text{....(2)}$$

$$Entropy = \sum_{i,j=0}^{N-1} -\ln(P_{i,j})P_{i,j} \quad \text{.....(3)}$$

$$Homogenity = \sum_{i,j=0}^{N-1} \frac{P_{i,j}}{1+(i-j)^2} \quad \text{....(4)}$$

$$Correlation = \sum_{i,j=0}^{N-1} \left(P_{i,j}\right) \frac{p(i-\mu)(j-\mu)}{\sigma^2} \quad \text{....(5)}$$

Where, $P_{i,j}$ – represents the element of Pixel at the coordinate position designated by i,j. It represents normalized symmetrical GLCM and it means the numeral of occurrences of grey scale i and j within the given window.

N - is the Total of grayscale in the image.

μ- is the GLCM mean.

σ^2 – is the variance of intensities contributed to GLCM.

The quantitative analysis of the biological activity of the sample is estimated by the fluctuations of speckle pattern intensity. The points in the central diagonal characterize no variation of intensity. But, the points spreading outside the diagonal denote the time-intensity changes. Slow variation of intensity according to the sluggish activity of the biological tissue and only noticeable values of the matrix appear nearby to the diagonal as depicted in Figure 3. In the high activity of bio speckle, intensity changes rapidly give high value from the principal diagonal of the matrix as shown in Figure 4.

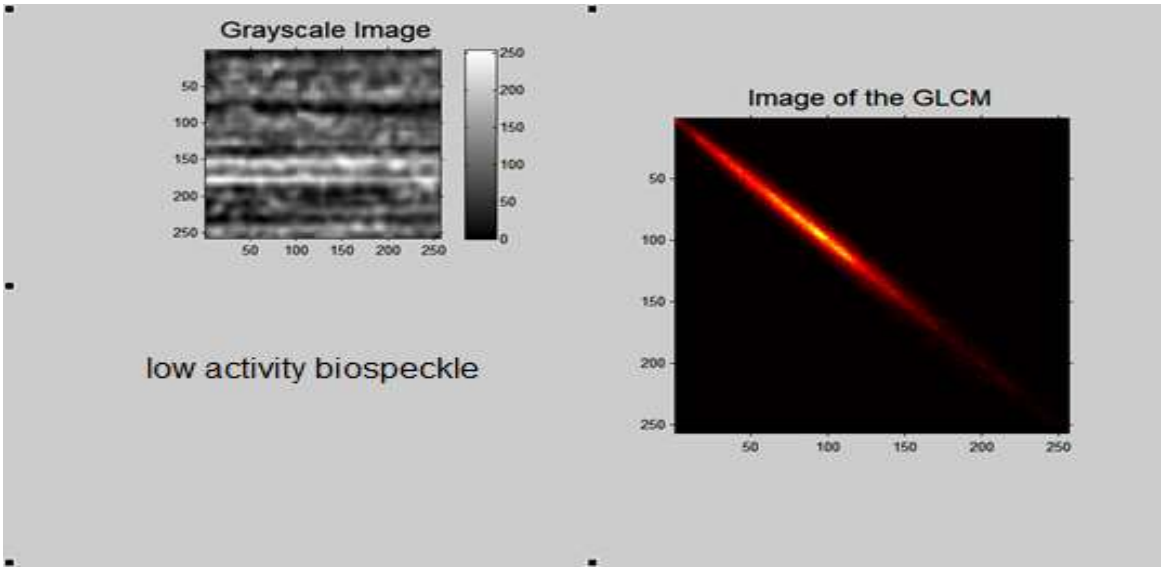


Figure 3. Bio speckle at the stage of Low activity (Final stage)

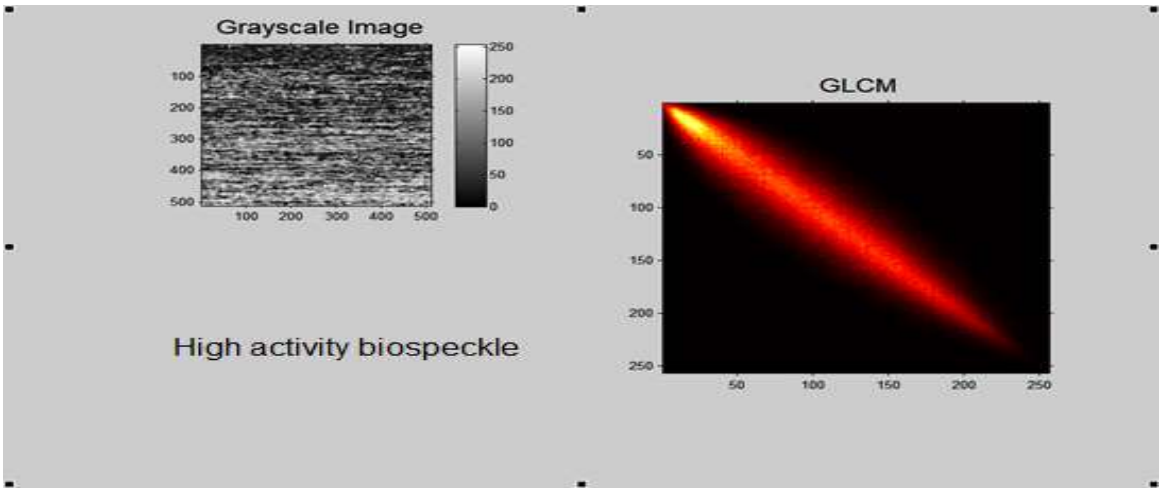


Figure 4. Bio speckle at the stage of High activity (initial stage)

No variation in intensity and correspondingly non zero matrices be appropriate to principal diagonal. Intensity changes with time according to the sample activity and hence number N increases outside the diagonal. The inside motion of the biological matter influences the intensity fluctuations in the results. During the low activity, the TASP displays a narrow straight line shape in Figure 3. At the time of high activity, TASP scattering more from its diagonal line is depicted in Figure 4. Therefore, it is established that the bioactivity of the sample decreases with time. Co-occurrence matrix (COM) is a good method to characterize the textural image features of biological activity of the sample shown in Figure 5

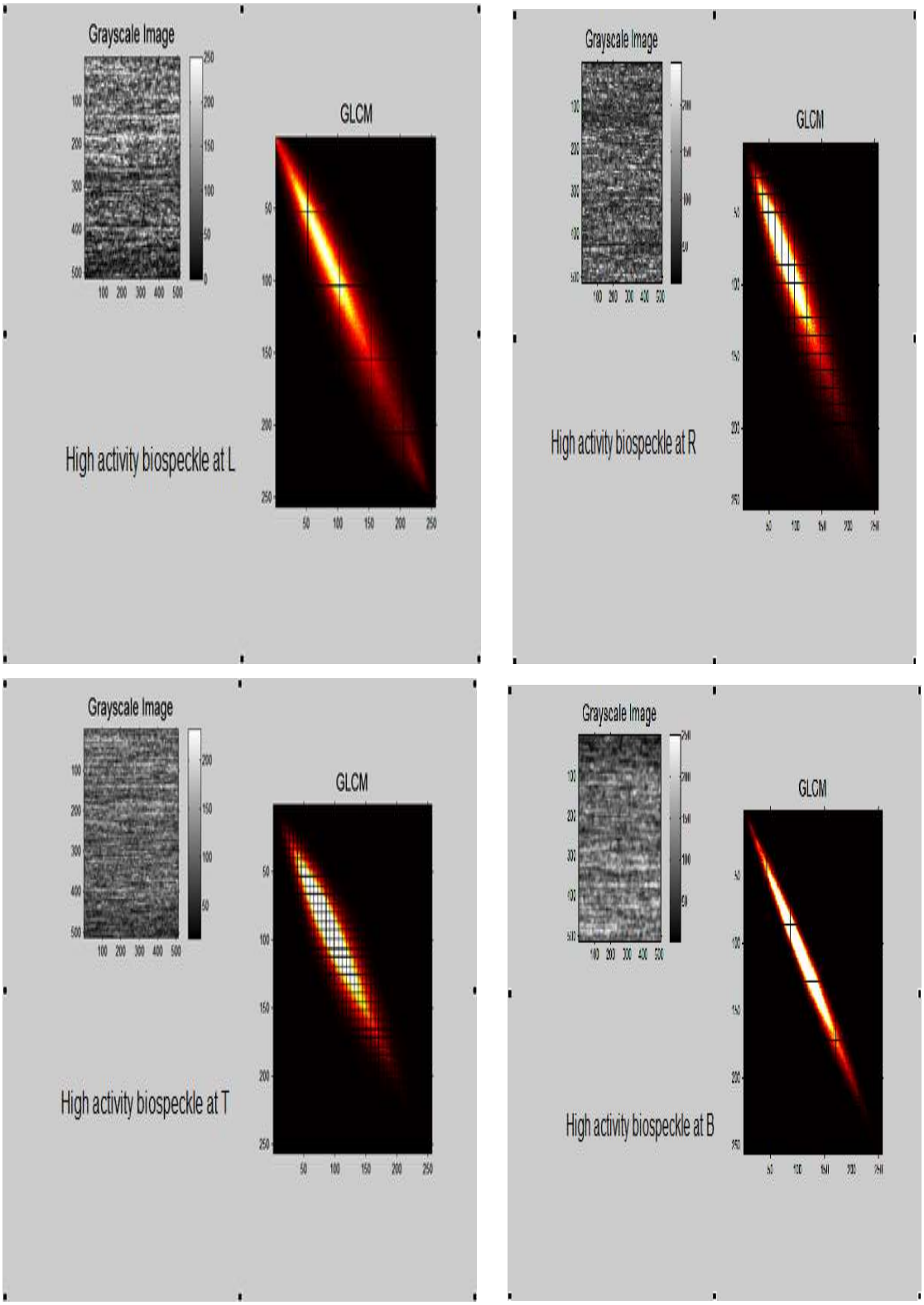


Figure 5. Bio speckle at different locations (L-R-B-T in clockwise)

Angle in degrees	Angular second Moment (or) Energy	Contrast	Correlation	Inverse different moment	Entropy
0	1.79E-04	124.496	3.88E-04	0.137	8.951
90	1.16E-04	305.000	3.74E-04	0.092	9.414
180	1.79E-04	128.464	3.88E-04	0.137	8.951
270	1.16E-04	289.452	3.75E-04	0.092	9.413

Table-1. Bio speckle High activity Elements

Angle in degrees	Angular second Moment (or) Energy	Contrast	Correlation	Inverse different moment	Entropy
0	9.65E-04	22.585	4.60E-04	0.482	7.339
90	5.53E-04	17.301	4.60E-04	0.305	7.890
180	9.66E-04	23.118	4.59E-04	0.482	7.340
270	5.53E-04	32.684	4.59E-04	0.305	7.891

Table-2. Bio speckle Low Activity Elements

The following facts are observed from the outcome of the image analysis:

1. Continuing reduction in cell activities at the post-harvest senescence of the fruit, makes the reduction of firmness because of loss of water and deterioration condition of the cell wall Angular second moment or energy value becomes high during the grey level distribution is almost constant in every angle. The high activity at 270˚ is 0.000116 (Table-1), but at the same angle in low activity, the value is 0.000553 (Table-2), which is a comparatively higher value due to constant energy at the final stage.

2. Laser penetrates deeply as fruits ripen due to the ruin of chlorophyll. Moving particles induces continuously by time variations of the diffused light arising from the fruits. This fluctuation phenomenon makes the speckle movement well known to biologists and is easily observed. At angle 0 degrees and the opposite side 180 degrees, and at 90 degrees and 270 degrees both high and low activity energies are the same as mentioned in Tables 1&2. Biological matter inside the cell moving with random direction at various speeds with the change of time.

3. There is a wide contrast variation at the high activity stage but minimum differences in the low activity bio speckle. High activity contrasts minimum 124 to maximum 305 value. But in the low bio speckle the range is from 17 to 32 only. This is due to the poor chlorophyll and water content.

4. Entropy means disorder and its values are high at the starting stage of bio speckle due to the high activity of microorganisms. But later the values are becoming small in the low activity by the poor movement of the particulates. It shoots from movements occurring inside the cells, which are of two categories:

(i) a continuous slow movement of the chloroplasts and amyloplasts (tiny particles of mean size about 1gm) in the cytoplasm medium.

(ii) an arbitrary movement of biological elements confined in cell vacuoles.

5. Bio speckle characterizes the living state of the cells. Correlation is challenging in the high activity bio speckle shown in Table-1 by its different values at various places and deviation is comparatively high. But the correlation is low activity at a different angle is more or less the same values. Hence, it is found that the ageing of an apple affects the correlation coefficient value and becomes constant because of no fluctuation in intensity and more tissues die. we can analyse each connected set of pixels in laser speckle images after converting them into binary images. The connected components are accomplishment in a binary image and produce a labelled image in which each pixel has the integer label of either 0 or 1. Healthy parts of the fruit show bright and bruise portion indicates the darkness. Bio speckle activity decreases with chlorophyll content was observed.

3.2. Roughness of the skin variation

High activity identifies the study of the nutrient transportation system and water content. The fading number of dark spots causes the increasing Ra values in different stages in Figure 6-8.

Integrity loss of cell wall components and cell turgor alters during fruit ripening leads to the loss of firmness. The specimen skin preserves the shape and reliability of the apple. It prevents microbial invasion and acts as a physical wall. The weight loss and change in roughness at the surface occurs during the maturing/senescence process. It is found that the roughness at the skin of the sample is related to the fruit water status. Roughness increases due to turgidity loss of the flesh than from drying up the surface underlying pericarp tissue. The sample skin surface contributes nearly 45% of the whole firmness of the specimen.

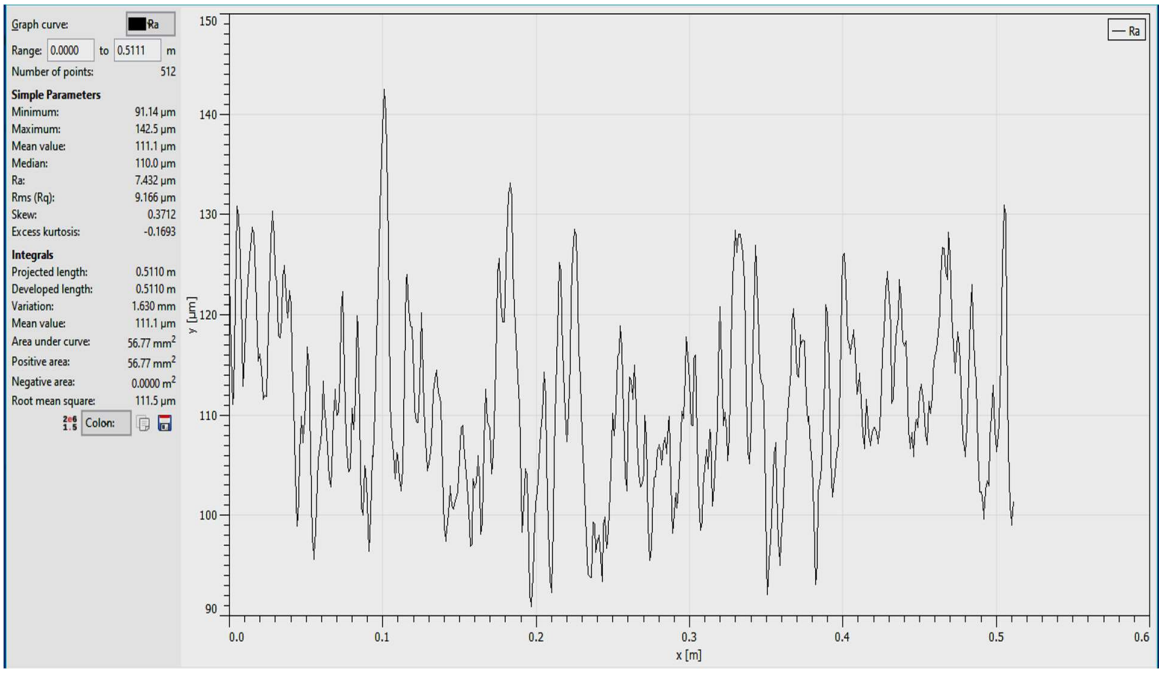


Figure 6. Roughness parameters of the biological sample – Initial stage.

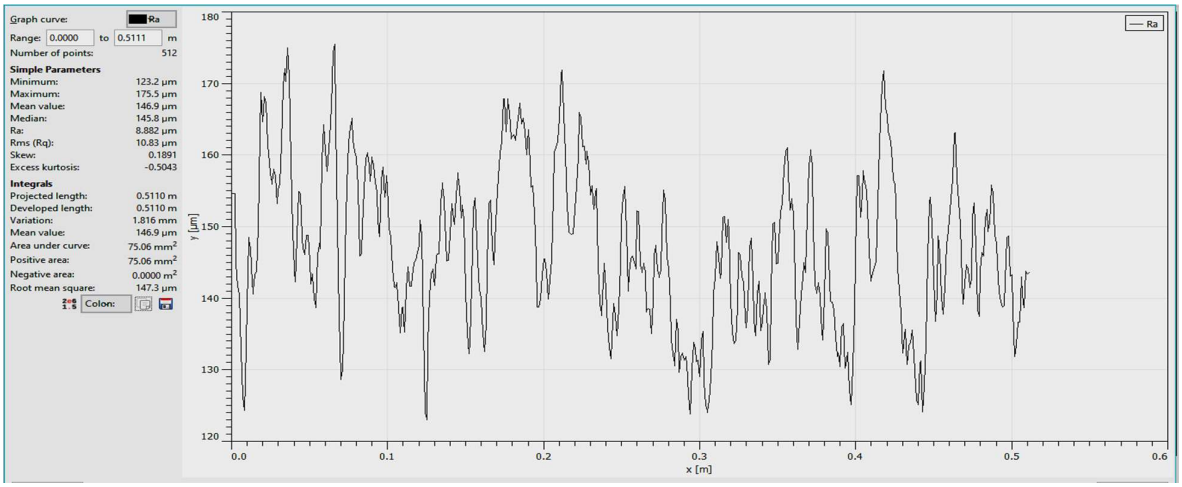


Figure 7. Roughness parameters of the biological sample – Middle stage.

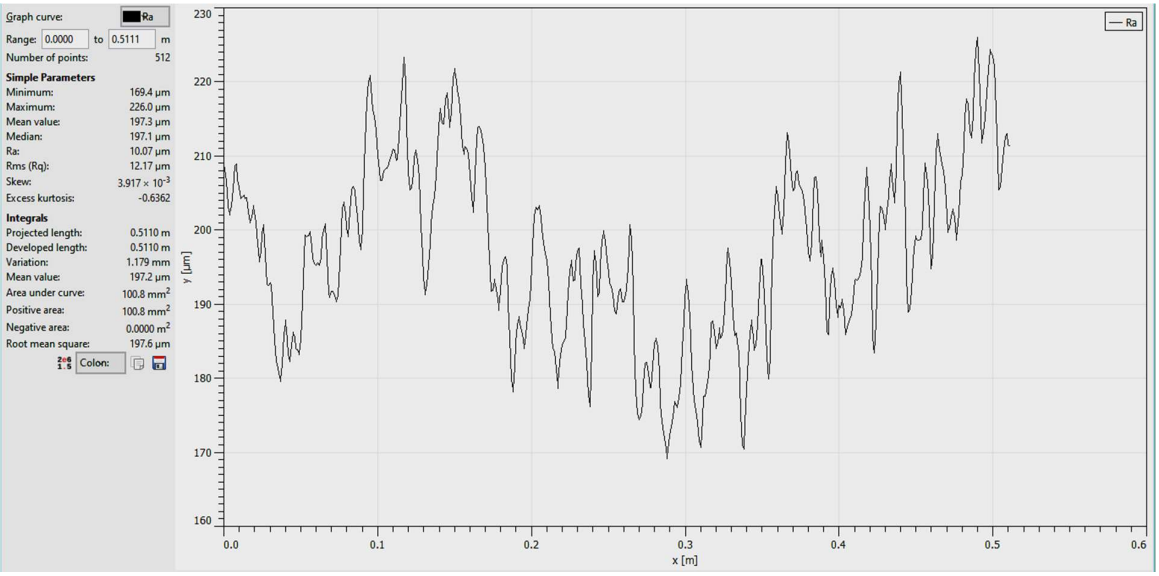


Figure 8. Roughness parameters of the biological sample –Final stage

3.3. Computation of Bright (B) and Dark (D) pixel

The bio speckle activity analysis shows its significance in the assessment of the ideal harvest window, the observing vegetables and fruit maturing process, and enlightening diseases and bruising. Colour and appearance, flavour (taste & aroma), texture morphological study and nutrition analysis are the most essential features for quality evaluation in fruits and vegetables. Hand-held puncture testers or penetrometers are the usual techniques for the evaluation of fruits.

These methods destruct the objects/specimen by using force and makes deformation to push a probe or punch into the specimen fruit to a depth that causes irreversible damage or failure. Many investigators found a correlation between resonance (acoustic or sonic, dynamic oscillation) on apple including sensory and destructive compression and puncture tests [12]. Bright (B) and Dark (D) pixel counting is a simple method of assessment in the surface roughness of the fruit as a textural calculation.

Bright pixels are obtained by constructive interference and the black portions are due to destructive interference. Maturation and ripening of natural pigments cause the colours of fruits. Fat-soluble chlorophylls provide green colour and carotenoid pigments develop yellow, red and orange colours. Water-soluble anthocyanins give red, blue, yellow colour is due to the presence of flavonoids and betalains contributes red reported in [13]. Enzym reactions create black, grey and brown colour pigments. Browning in apples is by reason of the oxidation of polyphenol compounds. The chlorophylls are affected by heat, but carotenoids are influenced by light and oxidation. Texture, consistency, rheology and viscosity are interchangeable conceptions. In

general, vegetables are in viscoelastic nature, hence they show both the properties of ideal liquids and solids, which demonstrates viscosity (flow), and elasticity (deformation) as reported by Bourne [14]. The freshness of the apple is indicated by its glow of colour and defects-free surface. Colour is characterized by defining lightness or the degree to which an object reflects light, and chroma or saturation, which is the intensity of colour or difference with the greyscale. Laser irradiation on each boundary area such as intracellular membranes, organelles and other particles without destruction by suitable intensity. Depending on the size of the scattered object in the living plant tissues are elastically scattered according to Rayleigh or Mie scattering. Unstable bio speckle fluctuating happens due to any entity in the target tissue moving by cytoplasmic streaming effect. So, bio speckle activity depends on the mobility of these particles.

The grayscale intensity in speckle images is related to surface roughness [15]. Binarized speckle images with all 0 or all 1 values of pixels are considered for this study wherever they are located (vertically, horizontally, or diagonally adjacent). The general structural information about the fruit cell wall can be obtained by the characterisation of polysaccharides in the nanoscale. There is a correlation between the area of white (B) spots and the surface roughness; both are increasing with time up to a certain extent. The high activity of the speckle pattern and its b/d pixel is illustrated in Figure 9&10. Similarly, low activity speckle image with b/d pictorial representation is depicted in Figure s 11&12. This speckle fluctuation is due to the Brownian movement of elements inside the cell, such as intracellular organelles like chloroplasts and amyloplasts. The movement of biological elements at a speed of 1 micrometre to 1mm/min [16]. It depends on the function of temperature, laser intensity and wavelength. A high rate of metabolic activity increased particle motion. Low bio speckle activity shows deceased or becoming extinct tissue, a measure of low metabolic rate.

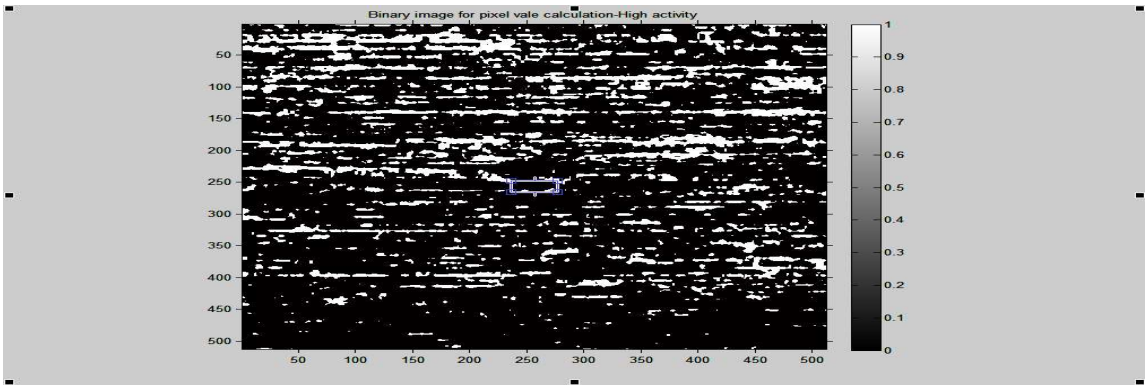


Figure 9. Binary image of Bio speckle activity (High)

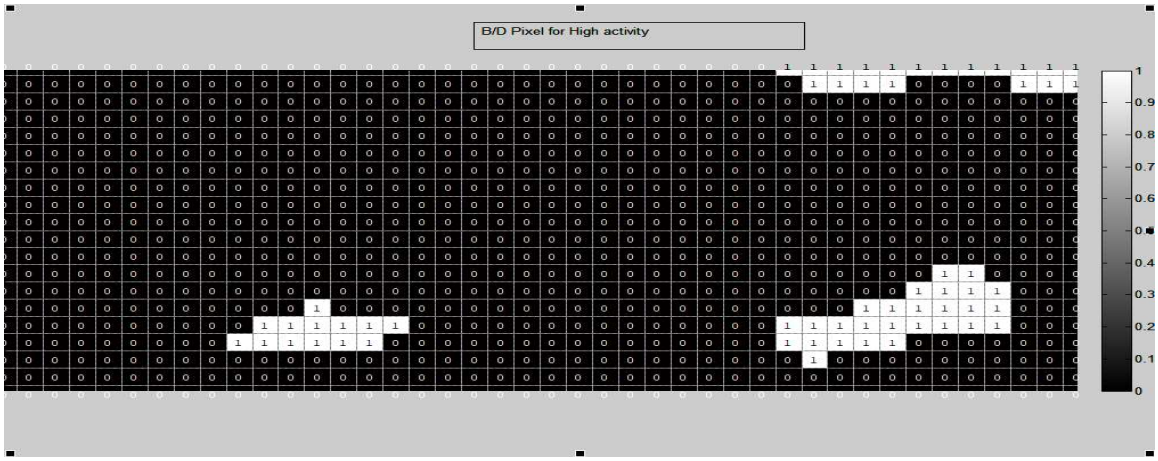


Figure 10. Binary image of bits B &D pixels for high activity

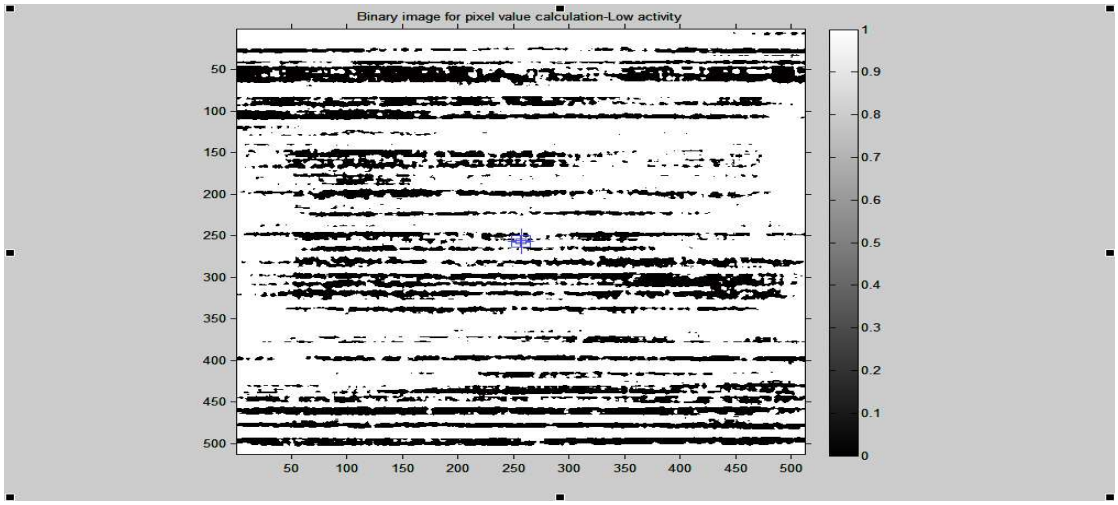


Figure 11. Binary image of Bio speckle activity (Low)

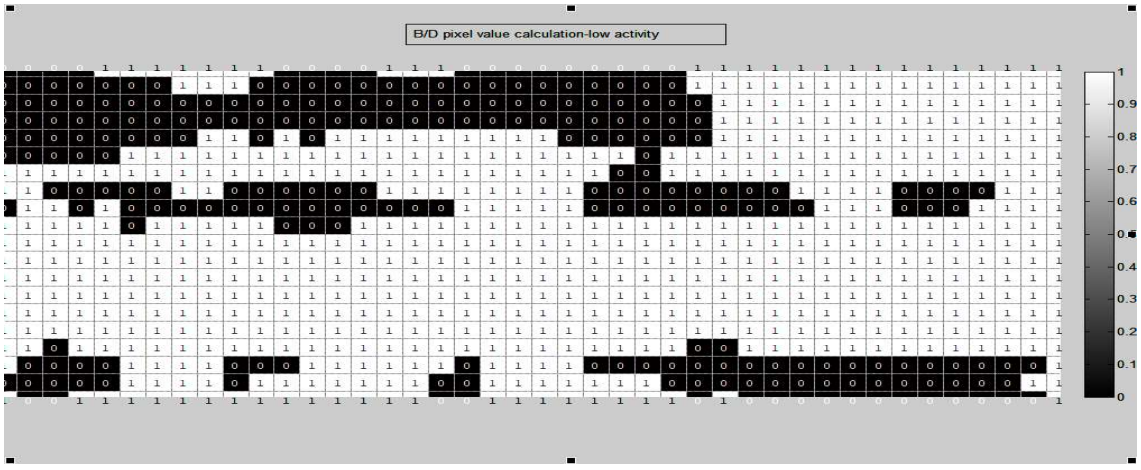


Figure 12. Binary image of bits B &D pixels for Low activity

CONCLUSION

This study highlights the effectiveness of a non-destructive bio speckle image processing technique for assessing apple maturation using a simple optical design. The method captures dynamic biological movements, allowing real-time monitoring of ripening phases. By employing the Grayscale Co-occurrence method and bright and dark (B&D) pixel counting, we gained insights into the physiological changes and surface roughness variations associated with different ripening stages. This comprehensive approach positions bio speckle imaging as a valuable tool for agricultural assessment and quality control in fruit production.

Conflict of interest: There is no conflict of interest.

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