Image processing technique for the Evaluation of Biological specimen using Laser speckle pattern

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Abstract

This work presents a study of biospeckle image correlation for the assessment of lemon fruit. This is a non-destructive and non-invasive optical technique. The study was carried out recording the temporal history of the speckle pattern obtained by illuminating the surface of the fruit with a laser beam. The biological activity of biomaterial has been inferred from the changes of intensity fluctuations with respect to time. These changes have been measured through correlation functions. Biospeckle analysis using Digital image correlation reflects the state of the investigated object.

Keywords: digital image correlation, non-destructive, biospeckle

1. Introduction

Digital speckle correlation techniques have been successfully proven to be an accurate analysis tool for a wide range of applications. Biospeckle is a technique which has its roots on Physics, mainly optical physics [1], [2], [3].The time varying speckle phenomenon has already been used to study a variety of objects that move in random ways. For example during the drying process of paint, the moving particles in it have been observed. The particles in the wet paint keep moving until the paint is completely dry [4]. The blood flow in superficial layers of skin has successfully been studied in [5], the activity of micro-organisms in a solution in [6] and the decadence of fresh fruit in [7].

The speckle techniques can be classified into three broad categories: speckle photography, speckle interferometry and speckle shear interferometry. Speckle photography technique has been adopted in this work for the assessment of lemon fruit.

2. Principles of Speckle Pattern

If a rough surface is illuminated by laser light, the light will be scattered back from every illuminated object point (Fig. 1). If the object is viewed by an eye or the camera, the object surface seems to be covered with bright and dark spots, which are called speckle. These speckles result from the path differences of the light emitted by the laser and reflected to the camera via different surface points.

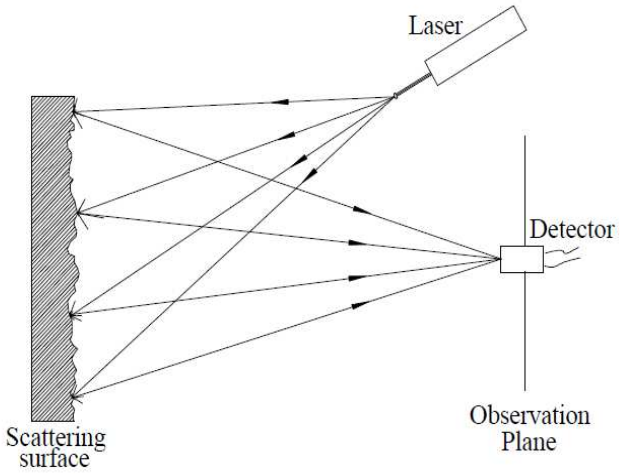

Fig. 1 Basics for Speckle image formation

When coherent light is incident on an optically rough surface, with height variations greater than the wavelength of the light, and is scattered from it, a pattern consisting of dark and bright spots known as speckles. The scattered waves interfere and form an interference pattern. This phenomenon is called the speckle effect. The speckle pattern is characterized by a random intensity and phase distribution. It is fundamentally a statistical process [8].

The intensity I is distributed according to the probability density function of a fully developed, polarized speckle field as follows:

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$$p(I) = \frac{1}{\langle I \rangle} \exp\left[-\frac{1}{\langle I \rangle} \right] \qquad I \geq 0 \qquad (1)$$

where I is the mean intensity value. The intensity I follows a negative exponential distribution (Fig. 2). Dark speckles are thus more likely, but there are always some very bright ones.

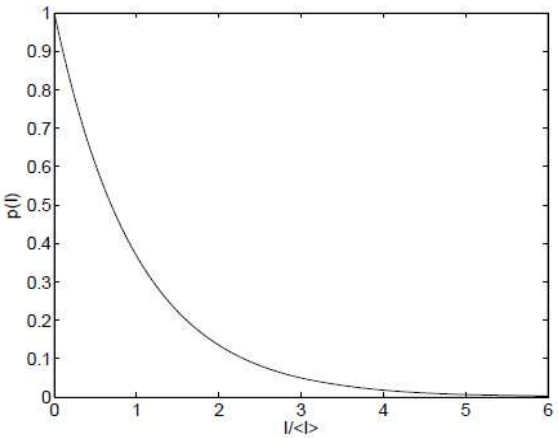


Fig. 2. Probability density of function of a polarized speckle.

If the statistical properties of the speckle pattern are determined by the size of the illuminated spot, the pattern is called objective. Instead if the statistical properties of the speckle pattern are determined by the aperture of the imaging system, the pattern is called subjective. For the case of a rectangular aperture the in-plane speckle width is defined as

$$\sigma_{x,y} = \frac{\lambda L}{D} \qquad (2)$$

where λ is the wavelength of the light, L is the distance between the aperture and the detector and D is the width of the rectangular aperture. The speckle length is defined as [9]

$$\sigma_Z = 7.31\lambda\left(\frac{L}{D}\right)^2 \qquad (3)$$

This means that the speckles have the shape of a cigar, since they have a larger size in the z-direction than in the x- and y-direction, unless for very large numerical apertures.

3. Biospeckle

The variable in time speckle pattern is characteristic for biological tissue and has been called as the biospeckle. Light interacts with matter in various ways (Fig.3). The different processes that occur depend on the wavelength of the light as well as the structure of the medium. Light can be reflected, scattered or absorbed when it interacts with the matter. Photons with very high energy, like gamma and x-rays, may even ionize atoms or break bonds in the molecules, but this will not be the case in this work since visible light is being used through all the experiments.

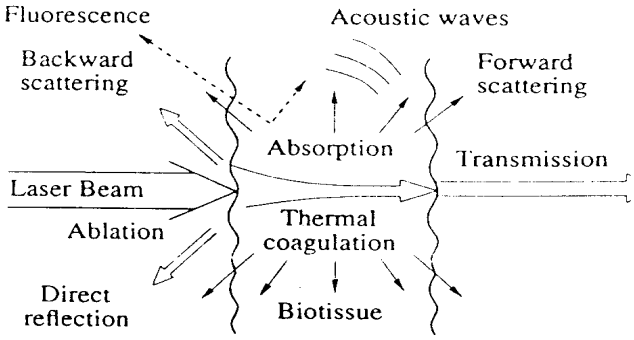


Fig. 3. Light interaction with matter

The reflection of the light, when it enters a border between different refractive indexes, obeys the laws of Snell and Fresnel. It depends therefore on the refractive indexes as well as the angle of the incoming and the reflected rays:

$$n_1 . \sin\varphi_1 = n_2 . \sin\varphi_2 \qquad (4)$$

$$R = \frac{1}{2}\left[\frac{\sin^2(\varphi_1 - \varphi_2)}{\sin^2(\varphi_1 + \varphi_2)} + \frac{\tan^2(\varphi_1 - \varphi_2)}{\tan^2(\varphi_1 + \varphi_2)} \right] (\text{Fresnel})) \qquad (5)$$

where n_1, n_2 are the refractive indexes in the two materials and φ_1, φ_2 the angles of the light perpendicular to the boundary. The scattering can be either elastic or inelastic. Elastic means that the scattered photons neither loose nor gain energy in the process. Brownian motions should be considered as a source of biospeckle activity too [10].

4. Experimental Setup

The biospeckle measurement device consists of simple low power diode laser (632 nm, 0.98 mW) with a microscope objective 10X as the beam expander to illuminate the sample. Biospeckle were recorded by a CCD camera. The camera-object distance maintained at a suitable distance to obtain the uncompressed images (BMP, 8 bits) with regular interval of time. Gain and brightness of the CCD camera were optimized experimentally, in order to avoid overexposed pixels on an image histogram. The image acquisition settings were kept unchanged during the whole experiment. Digital image correlation on the biospeckle activity of biomaterial has been carried out and the problem to be solved is the isolation of vibration.

5. Result and Analysis

The sample was exposed to the laser beam during a short period of time and the respective spatial temporal speckle pattern (STS) were obtained.

Fig. 4 Biospeckle (1 day)

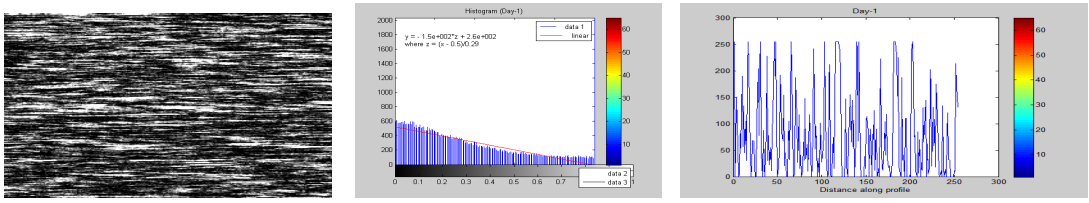


Fig. 5 Biospeckle (3 day)

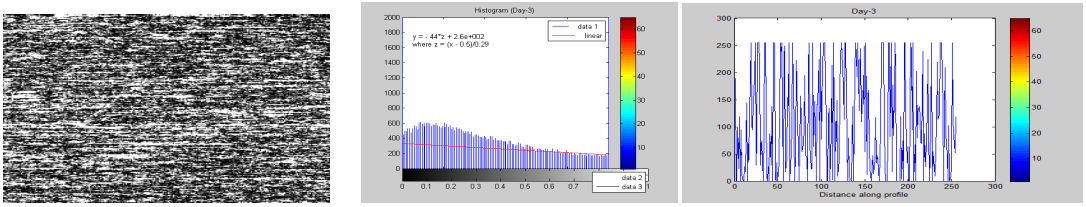
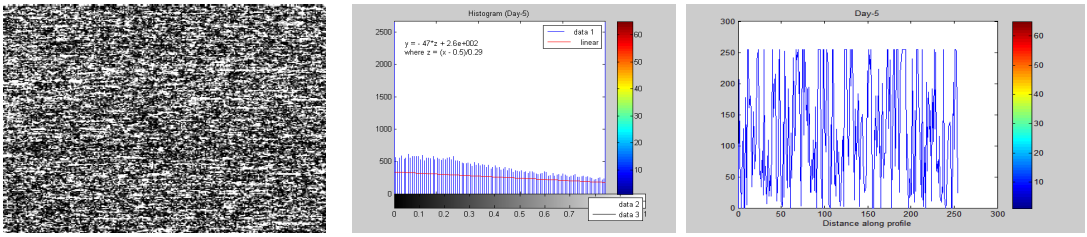


Fig. 6 Biospeckle (5 day)



Fourier transform low-pass filtering method is a form of low pass filtering in frequency domain. With this method, an image is transformed into a frequency domain and an appropriate window is selected in the frequency domain to eliminate the high frequency noise. The filtering is expressed as:

$$g(x, y) = FT^{-1}\{FT[f(x, y)]H(u, v)\} \qquad (6)$$

where FT represents Fourier transform, FT^{-1} represents inverse Fourier transform, H(u, v) is a filter function, f(x, y) is the original intensity and g(x, y) is the modified intensity. The intensity distribution of the fringe patterns can be expressed as:

$$I(x, y) = I_m(x, y) + I_a(x, y)\cos[\phi(x, y)] \qquad (7)$$

where I is the intensity distribution of the interferogram, I_m is the mean intensity, I_a is the intensity modulation amplitude, ϕ is the angular phase information of the interferogram, and (x,y) represents all the points in the x-y plane of the object and the interferogram. The intensity function can also be expressed as:

$$I(x, y) = I_m(x, y) + c(x, y) + c^*(x, y) \qquad (8)$$

$$c(x, y) = \frac{1}{2} I_a(x, y) e^{i\phi}(x, y) \qquad (9)$$

where * denotes complex conjugate. After a two-dimensional discrete Fourier transform (DFT), the spatial frequency domain representation of the pattern becomes

$$F(\zeta, \eta) = A(\zeta, \eta) + C(\zeta, \eta) + C^*(\zeta, \eta) \qquad (10)$$

where $A(\zeta, \eta)$ is the transform of Im(x, y), and $C(\zeta, \eta)$ and $C^*(\zeta, \eta)$ are the positive and negative frequency spectra of the modulated carrier fringes. ζ and η are the spatial frequencies that represent intensity changes with respect to spatial distances. If the image size is 2n, the fast Fourier transform (FFT) can be used, which is much faster than the ordinary Fourier transform. At the frequency domain, if $C(\zeta, \eta)$ can be isolated from $A(\zeta, \eta)$ and $C^*(\zeta, \eta)$, then an inverse Fourier transform can be performed for $C(\zeta, \eta)$.

Finally, c(x, y) can be obtained at the spatial domain and the phase information ϕ can be calculated from

$$\phi = \arctan \frac{Im[c(x, y)]}{Re[c(x, y)]} \qquad (11)$$

where Im[ ], Re[ ] represent the imaginary and real part of c(x, y), respectively. The phase ϕ obtained from the above equation ranges from $-\pi$ to $+\pi$ In order to be able to isolate $C(\zeta, \eta)$ from $A(\zeta, \eta)$ and $C^*(\zeta, \eta)$ at the frequency domain,

the intensity function should have continuous and monotonically changing derivatives across the field.

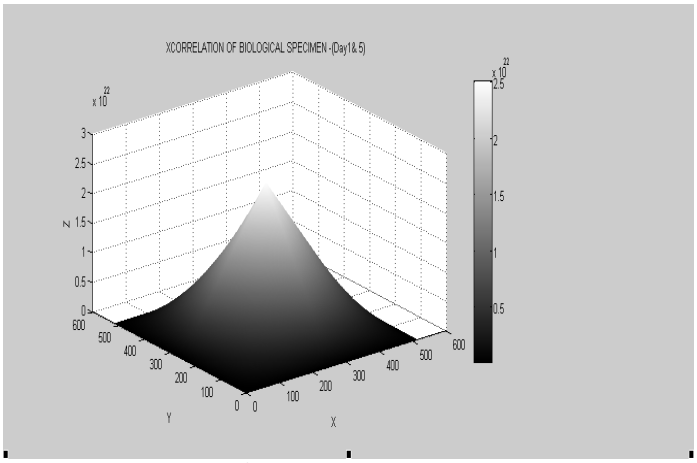


Fig. 7. Correlation of biological specimen based on time period

6. Conclusion

It is observed that the dynamic biospeckle pattern varies according to the change of physical composition of the tissue with time period. The time histories were correlated and Fourier transformed to obtain the frequency spectra of the scattered light of laser beam. The cross correlation coefficient of biospeckle patterns decreases along with Moment of inertia speedily when the specimen is fresh.

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