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# Exponentially smoothed Fujii index for online imaging of biospeckle spatial activity

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ABSTRACT

This paper describes a simple and efficient approach for the real-time evaluation of biospeckle spatial activity using a live video stream. The proposed method combines the exponentially weighted averaging of time-series data with a calculation of the Fujii biospeckle activity index. The exponentially smoothed Fujii method (ESF) was compared with the conventional offline method. A comparison was carried out using the speckle data of apple fruit with a fungal infection. Using data from a model experiment it was shown that the proposed method is capable of producing consistent and reliable results which are comparable with results obtained using the conventional approach. The exponentially smoothed Fujii method (ESF) provided high contrast maps of biospeckle activity without any loss of resolution. Reasonable computational demands made the practical implementation of this method possible using a standard desktop computer.



## 1. Introduction

Biospeckle imaging is a non-invasive technique based on an optical phenomenon which occurs during the illumination of a sample surface by coherent light from sources such as a laser. The wavefronts of the scattered rays interfere with each other and form random, granular patterns consisting of dark and bright spots, visible in the observation plane. The diffraction pattern depends on the geometry of the system, the wavelength of the laser and the aperture of the lens of the capturing device. In biological samples, the intensity of the biospeckle pattern evolves and fluctuates with time.

Draijer et al. (2009) suggested that this dynamic behavior is mainly caused by Doppler shifts of the light as it interacts with moving particles. It has been shown that processes such as cytoplasmic streaming, organelle movement, cell growth and division during fruit maturation and biochemical reactions are responsible for certain biospeckle activity (Kurenda et al., 2013). In addition to this, biospeckle fluctuations also depend on the chlorophyll (Romero et al., 2009) and starch content (Zdunek and Cybulska, 2011), as well as the temperature of the plant sample under investigation (Kurenda et al., 2012). To date, the applications of the biospeckle technique in agriculture include the determination of the quality and the degree of maturation of fruits and vegetables (Oulamara et al., 1989; Xu et al., 1995; Rabelo et al., 2005; Szymanska-Chargot et al., 2012), the analysis of seed viability (Braga et al., 2003), the detection of plant root bioactivity changes (Braga et al., 2009), the detection of fungal infection (Rabelo et al., 2011) or internal damage detection (Pajuelo et al., 2003).

Different techniques have been developed to measure the level of fluctuation of dynamic speckle. Arizaga et al. (1999) suggested an approach to characterize speckle time evolution based on the inertia moment (IM) of the co-occurrence matrix calculated from the time history speckle pattern (THSP), the spectral content of THSP lines was also analyzed by means of the coherence function, derived from cross-spectrum (Nobre et al., 2009), Passoni et al. (2005) proposed the wavelet entropy (WE) as a measure of the activity of dynamic speckle phenomenon, Zhong et al. (2013) proposed a normal vector based approach as a new descriptor of time-varying speckle pattern to address this issue, Cheng et al. (2003) proposed a technique in which the velocity information coded in speckle pattern was obtained through first-order temporal statistics of a time-averaged speckle image, Martí-López et al. (2010) proposed and tested temporal difference method for processing dynamic speckle images. A spatial dynamic speckle pattern occurs when a large enough area is illuminated by an expanded laser beam. In general, these techniques can be divided into two groups – global activity measurements and spatial activity measurements. Global measurements provide a single activity index calculated using data from a small region of the sample. When a relatively large area is observed, such speckle patterns depict the spatial

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variability of speckle fluctuations that correspond to the underlying dynamic processes. Speckle activity can be presented by means of spatial activity maps, that is, locally calculated measurements assigned to specific locations.

At the present time, the literature describes a wide range of methods for spatial speckle image analysis. However, only a few can be implemented for real-time processing. One of the first and most popular methods of speckle analysis is known as LASCA - Laser Speckle Contrast Analysis (Briers et al., 1995; Briers, 2007). Two alternative measurements of speckle contrast analysis were proposed - spatially averaged and time-averaged contrast analysis (Briers, 1975, 1978). While time-averaged contrast has high computation costs and requires a database of historical images, the spatially averaged variation of this method has been successfully employed for real-time processing. However, the major drawback of this method is that it reduces the effective resolution of the processed images.

Godinho et al. (2012) proposed a feasible alternative to the routine online methods based on the Motion History Image (MHI) methodology. The MHI technique was capable of maintaining the resolution and the definition of the original images. This technique produced better results, when compared to the online version of LASCA, but less favourable results compared to offline methods. Another popular measurement used to evaluate speckle spatial activity is the Fujii index, which was originally applied to observations of blood flow (Fujii et al., 1985, 1987). This technique is known to provide high quality images, with high contrast and without loss of resolution. However, this method requires data-intensive computing therefore online imaging is difficult when using a basic approach.

In this paper we present an exponentially smoothed Fujii method (ESF) for the real-time processing of biospeckle spatial activity using a live video stream. Our approach comprises an exponentially weighted moving averaging of time-series data with the calculation of the Fujii biospeckle activity index. The ESF method was compared with the conventional offline approach as well as with the MHI method using experimental data showing fungal infection of apple fruit. Finally, a practical implementation was introduced to demonstrate the capabilities of the exponentially smoothed Fujii method for real-time processing.

## 2. Materials and methods

### 2.1. Experimental setup

The proposed method was tested on a suitable sample of ten apple fruit (*Malus Domestica*, cv. "Golden Delicious") infected with *Pezicula malicorticis* (H. Jacks.) Nannfeld fungal cultures. *Pezicula malicorticis* is an apple fruit rotting fungi which causes the necrosis of fruit flesh described in the literature as anthracnose canker and bull's-eye rot. Healthy apples were infected through an injection of 0.2 ml of a solution of fungal cells using tuberculin syringes with a capacity of 1 ml and injection needles with a diameter of 0.4 mm. The concentration of cells in the water solution was determined using a Thoma chamber and was equivalent to $1.2 * 10^2$ cells/ml. The apples were incubated at 22 °C. The biospeckle activity of the apples was recorded after 3 days of disease development. Fig. 1 shows an example of apple fruit with developed fungal infection.

The biospeckle experimental setup was similar to that which was used in our previous studies (Zdunek and Cybulska, 2011). Apple fruit was illuminated using a diode laser (1000 mW, $\lambda$ = 532 nm, SDL-532-1000 T, with power supply SDL-PS-500, Shanghai Dream Lasers Technology Co., Ltd., Shanghai, 201611, China), coupled to a 20x beam expander (Edmund Optics GmbH, Karlsruhe, Germany). The biospeckle phenomenon was captured using a CCD camera (monochrome BASLER acA2000-340 km camera, Basler AG, Ahrensburg, Germany) with a 25 mm TV lens 1:14 and 20 mm extension ring (Pentax Corporation, Tokyo, Japan). The camera–apple distance was about 150 mm and the laser–apple distance was 500 mm. The illumination angle was $\theta \approx 30°$. The camera captured uncompressed, 8-bit, video sequences at 60 frames per second, lasting 10 s. The exposure time was equal to 2000 µs, gain and gamma were set experimentally to cover as much as possible of the dynamic range of the camera and avoid pixel over and underexposure. The image resolution was 640 × 480 pixels, which corresponded to an observation area of about 40 mm × 30 mm.

### 2.2. Analysis of biospeckle spatial activity

#### 2.2.1. Offline Fujii method

The Fujii method is based on a calculation of the weighted sums of the absolute differences in gray-level intensity for each pixel of the time series of dynamic speckle patterns. The value of the Fujii index at each specified location is defined as:

$$F(x,y) = \sum_{k} \frac{|I_k(x,y) - I_{k+1}(x,y)|}{I_k(x,y) + I_{k+1}(x,y)}$$

where $k$ is the image index from the time series $k = 1 \ldots N$, $I_k$ and $I_{k+1}$ are the intensity values of a pixel at a location given by coordinates $x$ and $y$.

The presence of the weighting factor in the denominator of the Fujii index results in a nonlinear response, which in turn results in amplified speckle activity in darker, less illuminated regions of the speckle pattern. Two additional parameters were introduced to resolve this issue – $t_d$ and $t_s$, which are the absolute difference threshold value and gray levels sum threshold value respectively.

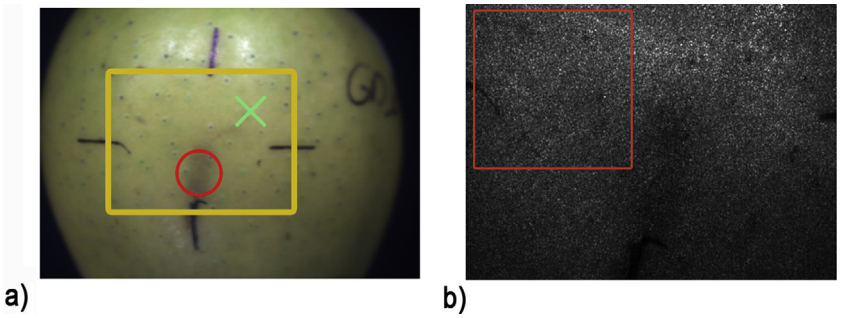


**Fig.1.** Image of infected apple with marked region of interest (ROI), infected (red circle) and healthy (green cross) region of apple (a), biospeckle image of ROI (b) with a marked rectangular region (red square) used for calculation of image statistics. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)