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Dynamic speckle technique as a leaf contamination sensor

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The phenomenon of laser speckle yields information about physical, chemical or biological activity in time for various objects (e.g. fruits, seeds, coatings) through statistical description of speckle dynamics. The paper introduces analysis of dynamic speckle patterns as an effective leaf contamination sensor by non-destructive whole-field characterization of the tested samples with high spatial and temporal resolution. More specifically, the paper presents the results of two test experiments in order to detect any variation of activity related to increase of humidity or treatment by chemical agents. In total, time sequences of 256 images of speckle patterns were recorded and processed by pointwise implementation of correlation-based algorithms. The experiments proved the ability of this approach to differentiate between differently treated leaves.

Keywords: dynamic speckle, leaf contamination, correlation analysis

INTRODUCTION

Coherent illumination of a diffuse object yields a randomly varying speckle pattern[1] in case of physical, chemical or biological activity within the object. This phenomenon can be used for non-invasive whole-field detection and visualization of processes in biological samples through statistical description of laser speckle dynamics [2]. The main advantage of this approach is ability to perform distant measurements with high spatial and temporal resolution without requirement for sophisticated equipment. Speckle fluctuations can be easily seen with a bare eye but a comprehensive statistical analysis is required to retrieve relevant information [3]. Usage of modern 2D optical sensors to register time sequences of speckle patterns provides large amount of data for accurate estimation of both first and second statistical moments of the recorded intensity data [1, 3] that can be used to characterize activity of samples. Dynamic laser speckle has been applied to study perfusion of blood flow in human tissues in medicine [4-5], bacterial response in biology [6], plant development processes [7], seeds viability [8], as well as for quality assessment of fruits and control of pasty products [11-13].

Here we reported the results of test experiments conducted to prove the efficiency of dynamic speckle technique for detection of changes on a leaf surface after applying a chemical agent. For the purpose, randomly varying speckle patterns of laser light, reflected from the leaf surface, were recorded as time sequences. They were used to calculate spatial distributions of temporal correlation and structure functions across the leaf surface and to evaluate the undergoing activity in time. The results proved the ability of this approach to differentiate between differently treated leaves.

EXPERIMENTAL AND DATA PROCESSING

In the set-up for acquisition and storage of dynamic speckle patterns (Fig. 1) an expanded beam from a He-Ne laser (632.8 nm and 3.2 mW) illuminated the leaf sample placed on a horizontal stage through a ground glass diffuser. The set-up was positioned on a vibration insulated table. An optical axis of a CCD camera, adjusted to focus the sample, was normal to its surface. The camera recorded regularly a sequence of 8-bit encoded images at sampling frequency chosen to provide at least 10-20 points within the width of the temporal auto-correlation function which characterized intensity fluctuations due to sample activity.

We made two test experiments. In the first one, a pea leaf was placed on a sheet of paper. Two separate acquisitions of speckle patterns were carried on under different humidity conditions. In the second experiment three leaves (Tradescantia

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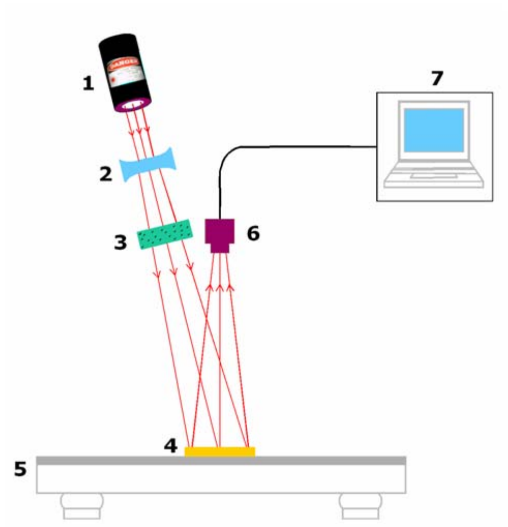

Fig. 1. Set-up for acquisition and storage of dynamic speckle patterns: 1 – He-Ne laser, 2 – beam expander, 3 – ground glass diffuser, 4 – sample, 5 – vibration insulated table, 6 – CCD camera, 7 – PC.

albiflora) were placed next to each other in a petri dish. Two drops of acid (HNO_3, 0.01%) and two drops of alkali (NaOH, 0.01%) water solutions were squeezed at the cut endings of the stems and at the middle parts of two of the leaves respectively; no chemical agent was applied to the third leaf. The leaves were fixed by sticking their tips with a plastic tape to the petri dish. The stem ending and the tip of the leaf without treatment were also fixed to the dish by a plastic tape to prevent penetration of the chemical agents from the other leaves.

Specifics of the observed object required to estimate activity at each point of the leaf surface. If N patterns of size $N_x \times N_y$ are acquired for time T at a sampling rate $1/\Delta t = N/T$ and a pixel period Δ, $N_x \times N_y$ time sequences of 8-bit encoded intensities $I_{kl,n} \equiv I(k\Delta, l\Delta, n\Delta t)$, $k = 1,2 \ldots N_x$, $l = 1,2 \ldots N_y$, $n = 1,2 \ldots N$ are formed. They allow for building a point wise estimate of a given statistical measure by averaging over *T*. We processed the data by correlation based algorithms which yield a set of 2D activity maps at increasing time lags and spatial resolution limited by the pixel period. We assume that the short-time activity within the sample is described by the 2D spatial distribution of the normalized temporal correlation function (NTCF), which is a function of the time lag, $\tau = m\Delta t$ at a point with spatial coordinates (x, y) and has a radius of correlation $\tau_c = \tau_c(k\Delta, l\Delta)$ which may vary from point to point. The estimate $\hat{R}_{norm}$ of R_{norm} is calculated from the series $I_{kl,n}$ as

$$\hat{R}_{norm}(k,l,m) = \frac{\chi_m}{\hat{\upsilon}(k,l)} \sum_{n=0}^{N-m} \left(I_{kl,n} - \bar{I}_{kl}\right)\left(I_{kl,n+m} - \bar{I}_{kl}\right) \tag{1}$$

with $\chi_m = \dfrac{1}{N-m+1}$ and $\hat{\upsilon}(k,l) = \dfrac{1}{N}\sum_{n=1}^{N}\left(I_{kl,n} - \bar{I}_{kl}\right)^2$, $\bar{I}_{kl} = \dfrac{1}{N}\sum_{n=1}^{N} I_{kl,n}$, where $\hat{\upsilon}(k,l)$ and $\bar{I}_{kl}$ are the estimates of the variance and the mean of the intensity fluctuations in time at the point $(k\Delta, l\Delta)$.

Theoretically R_{norm} gives degree of correlation and decreases with the time lag $\tau = m\Delta t$. Fully correlated state with zero activity corresponds to 1. The point wise processing yields a set of 2D spatial maps of activity at increasing time lags starting from $\tau_1 = \Delta t$ and going up to $\tau_M = M\Delta t$ with $M < N$. Although these maps are obtained for the averaging time interval *T*, they provide information about temporal scales of activity within this interval and in the different spatial regions of the sample. Using normalized correlation-based algorithms ensures spatial characterization of activity that is independent of illumination and reflectivity variation across the object surface.

RESULTS AND DISCUSSION

The results from the first test experiment are shown in Fig. 2 and Fig. 3, which give 2D gray-scale maps of the NTCF at four different lags for a pea leaf at normal (Fig. 2) and increased (Fig. 3) humidity. All maps were obtained after averaging over a sequence of N = 170 images with $N_x \times N_y$ = 600×500; the gray scale varies from -0.25 to 1.0. The intensity for the leaf region in the speckle patterns is considerably lower than for the surrounding sheet of paper, but the latter exhibits practically zero fluctuations with exception of the shadow zone. The shadow in Fig. 2 is cast by the stem of the leaf whereas in Fig. 3 it is caused by one of the leaf petals. Observation of activity in this zone is an indicator of microscopic changes of the leaf surface. As a whole, more or less uniform activity across the leaf surface is observed. As it should be expected, the processes within the leaf sample are going much faster and the correlation time is much shorter at increased humidity.

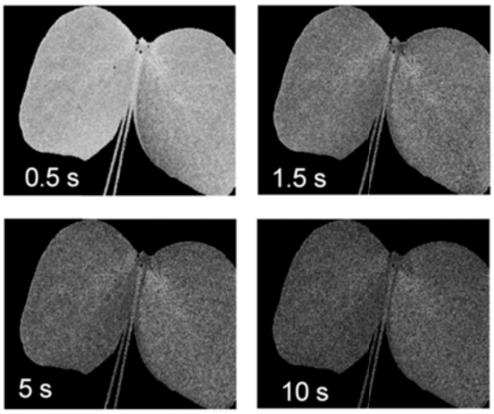

Fig. 2. Gray scale maps from -0.25 to 1 of a normalized temporal correlation function of speckle fluctuations of a pea leaf at different time lags between the processed patterns at normal humidity.

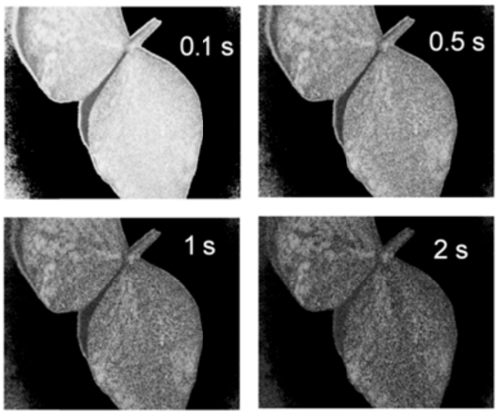

Fig. 3. Gray scale maps from -0.25 to 1 of a normalized temporal correlation function of speckle fluctuations of a pea leaf at different time lags between the processed patterns at increased humidity.

In the second experiment, fluctuations in intensity were observed also on the surface of the petri dish around the leaves due to its inevitable wetting by gradual spreading of the drops of acid and alkali solutions around and beneath the treated leaves. The reflectivity of leaves was much lower in comparison to the surrounding area. This means that the spread of speckle fluctuations in the region of the leaf is also lower than in the remaining part of the petri dish. As has been mentioned above, using of a normalized estimator solves the problem with the signal-dependent variance in a speckle pattern. To focus on the changes only in the region of interest – the surface of the leaves – we introduced a threshold value of intensity and calculated a binary mask which kept only the intensities below the threshold. We processed 7 sequences of 256 images that were recorded within two days at Δt =1.5 s. We noticed a substantial change in mean intensity on the second day that could not be explained only by increase of intensity within the laser spot. Figure 4 presents two distributions of the mean value estimate $\bar{I}_{kl}$ after averaging over 256 speckle patterns. The moments of recording the time sequences for both presented maps differ with 20 hours. The first map shows strongly varying reflectivity across the leaf samples, the intensity range is practically the same for the three leaves and the drops of the acid and alkali solutions at the middle part of the treated leaves are clearly seen. In Fig. 4 the leaf without

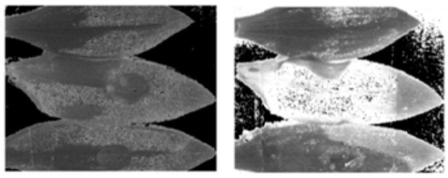

Fig. 4. Gray-scale maps from 0 to 100 of the mean intensity distribution calculated from 256 8-bit encoded speckle patterns at 0 h (left) and 20 h (right) after picking the leaves; top leaf – without treatment, middle leaf – with two drops of acid water solution, bottom leaf – with two drops of alkali water solution.

the treatment is at the top, the leaf treated with an acid solution – in the middle, and the leaf treated with alkali solution – at the bottom of the map. After 20 hours the reflectivity of the leaf with the acid treatment strongly increases all over its surface with exception of the area in the proximity of its fixed tip, and the contours of the drop remains only on the leaf with alkali treatment. Characterization of activity in this experiment was made by using a NTCF. To visualize better the spread of fluctuations in different regions we used a temporal structure function estimated as

$$\hat{S}(k,l,m) = \chi_m \sum_{n=0}^{N} \left(I_{kl,n} - I_{kl,n+m}\right)^2 \tag{2}$$

The results for N = 256 and for four of the acquired sequences of speckle patterns are shown in Fig. 5 where the first two columns give the NTCF maps whereas the other two – the structure function maps at 3 s and 6 s time lags respectively. The first row corresponds to the sequence of images recorded immediately after picking the leaves. According to the NTCF the activity close to the stems and inside the drops with chemical agents is higher. One hour later (second row in Fig. 5) the maps corresponding to the acid treatment show the

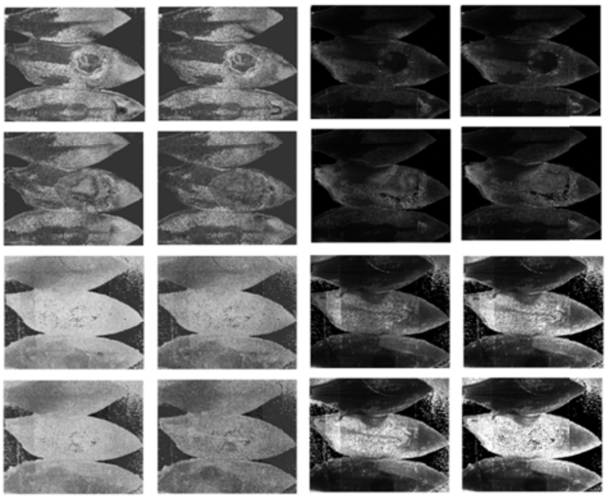

Fig. 5. Gray-scale maps from -0.25 to 1 of NTCF at time lag 3 s (1st column) and 6 s (2nd column); gray-scale maps from 0 to 60 of a structure function at time lag 3 s (3rd column) and 6 s (4th column). The maps are calculated from time sequences of 256 8-bit encoded speckle patterns acquired at 0 h (1st row), 1 h (2nd row), 20 h (3rd row), 21 h (4th row) after picking the leaves.

most considerable change: the drop is spread to a larger area in which reflectivity increases. The third and the fourth row in Fig. 5 present the same maps on the next day. The activity strongly decreases and has more uniform distribution. The leaf with the acid treatment shows less variation in time and much higher reflectivity than the other two leaves. The drop with alkali solution is still traceable as a zone of increased activity for the third leaf.

CONCLUSION

In summary, we checked the ability of dynamic speckle technique to detect changes on the leaf surface as a result of humidity variation or application of a chemical agent. We obtained encouraging results from the performed test experiments with point wise correlation-based algorithms. The calculated 2D gray-scale maps of temporal correlation or structure functions visualize the activity across the surface of the sample and indicate clearly the regions that have undergone different treatment.

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АНАЛИЗ НА ИЗМЕНЯЩИ СЕ СПЕКЪЛ КАРТИНИ КАТО НЕРАЗРУШАВАЩА СЕНЗОРНА ТЕХНИКА ЗА ЗАМЪРСЯВАНЕ НА ЛИСТА НА РАСТЕНИЯ

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(Резюме)

Явлението лазерен спекъл позволява проследяване на скоростта на протичане на физични, химични или биологични процеси в различни обекти като например плодове, семена, покрития и други чрез статистическо описание на динамиката на спекъл картината върху повърхността на тези обекти. Настоящата работа въвежда анализа на изменящи се във времето спекъл картини като неразрушаваща сензорна техника за замърсявания на листа на растения. Характерзирането на образците се извършва паралелно по цялата им повърхност с висока пространствена и времева разделителна способност. По-конкретно работата представя резултатите от два тестови експеримента за установяване на промяна в скоростта на процесите вследсвие на изменение на влажността и на химично въздействие. Като цяло, серии от 256 изображения на спекъл картини се записват последователно във времето и се обработват чрез корелационно-базирани алгоритми, прилагани за всяка точка от образеца. Експериментите потвърждават потенциала на този подход за различаване на листа, подложени на различно въздействие.