Application of statistical interferometry to monitor biological activity of plant under environmental stress

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ABSTRACT

In this study, a novel optical interferometric technique called 'statistical interferometry'¹⁻³ has been developed. In contrast to the conventional interferometry where the phase is determined in a completely deterministic way, we consider the interference of completely random wave fronts, i.e., speckle fields, and it has been proved that the complete randomness of the speckle field can play the role of a standard phase in a statistical sense. The advantage of the method is that since the phase of the object under testing can be derived in a statistical way, the accuracy of the measurement depends only on the number of data taken to calculate a probability density distribution of speckle phase. This feature permits a simple optical system to achieve measurements with an extremely high accuracy. According to a computer simulation, the accuracy of $\lambda/1000$ can be achieved using 40,000 data of the speckle intensity.

Statistical interferometry was applied to monitor biological activity or growth rate of plant, aiming to investigate the influence of the environmental pollutions. In the experiments, the plants were exposed to ozone that is the main substance of photochemical oxidant, and the growth rates were measured before and after the exposure. It was clearly observed that the fluctuation of growth rate as well as its mean rate was dramatically affected by the exposure of ozone. By the observation of growth rate of plant with the accuracy of sub-nanometer scale and a time scale of second, it was newly revealed that the fluctuation of the growth rate reflects the biological activity of the plant.

KEY WORDS: Statistical interferometry, Environmental pollution, Ozone, Plant growth

1. INTRODUCTION

Recently, rapid advancement in the human activities, especially in the industry, has led to serious problems of environmental pollution. One of the most significant effects of the environmental pollution is the acid rain that leads to the deterioration of the leaves of the trees. Therefore, demands to establish new techniques that can estimate the level of the damage to the plants has been on the rise.

In this study, we have developed an optical method to monitor the condition of plant, biological activity or growth, aiming to estimate the environmental conditions. For this purpose, the statistical interferometry has been applied to measure the growth of the plant. In our previous studies¹⁻³, the technique has been proposed and developed to measure the in-/out-of-plane deformations, and strain of non-biological object such as metal. The method is based on the complete randomness of the speckle phase in a fully developed speckle field, and it can play the role of a standard phase

in a statistical sense. The phase of the object under testing can be derived in a statistical way, in contrast to the conventional interferometry. This method has the advantage of its simple optical system to achieve measurements with an extremely high accuracy.

First, to demonstrate the validity of the present method, measurements of the growth speeds of the plants were performed under various environmental conditions such as the light illumination and the atmospheric pollutant.

2. OPTICAL SYSTEM AND THE PRINCIPLE OF STATISTICAL INTERFEROMETRY

Figure 1 illustrates the optical system employed to measure the growth of plants. A YAG (SHG) laser of wavelength $\lambda=532\text{nm}$ was used as a light source. The laser light is divided into two beams by means of the polarization beam splitter (PBS), and two beams are reflected by the mirrors M_1 and M_2 . These two beams are made parallel by means of the lens and illuminate the object normally. Two independent speckle fields

are generated from the illuminating points on the object. The separation d of the two beams is variable by setting the angle of the mirror M_1 . A CCD camera is arranged to observe the interference pattern of two speckle patterns at the angle θ from the illumination to the object. Since the orientations of polarization of the two illuminating beams are orthogonal, a polarizer (PL) was inserted. When the plant grows between the two illuminating points by a distance Δx , this introduces a phase change Ψ between the interfering speckle fields given by

$$\Psi = (2\pi / \lambda)\Delta x \sin \theta \quad (1)$$

The object phase Ψ is derived according to the algorithm of the statistical interferometry. We briefly explain the principle. As conventional interferometric techniques that permit high accuracy of the measurements, the phase shifting interferometry (PSI), heterodyne interferometry etc. are well known. In contrast to such a conventional and deterministic interferometric technique, we have taken a reversed approach to achieve a technique with high accuracy and have adopted the complete randomness of the interfering fields as a standard of phase in a statistical sense. The fully developed speckle field that can be easily generated from an optically rough surface has a completely randomized wavefront. This can be seen from the well known fact that the probability density function (PDF) of the speckle phase in the fully developed speckle field takes uniform distribution over $-\pi$ to π^4 . In our previous study, we pointed out that this randomness of the fully

Fig.1 Optical system to measure the growth of the plant. Two parallel laser beams illuminate normally the object, and the extension between the illuminating points is measured.

Fig.2 Effects of symmetrical $\Delta\Psi_s$ and antisymmetrical $\Delta\Psi_a$ phase terms on the probability density distribution of the evaluated speckle phases. (a) $\Delta\Psi_s=2\pi/50$ and (b) $2\pi/100$, and antisymmetrical phase term for (c) $\Delta\Psi_a=2\pi/100$ on the PDF $p_\phi(\phi')$ of evaluated speckle phase ϕ' . $p_\phi(\phi')$ becomes uniform when (d) $\Delta\Psi_s=\Delta\Psi_a=0$.

developed speckle field can be a standard for phase and proposed a statistical interferometry. In the method, random speckle interference patterns are successively taken as the object is deforming. We first assume an arbitrary value for the object phase to be determined, and then, speckle phase are next calculated from any combination of three frames of the speckle pattern. We refer this speckle phase as ‘evaluated speckle phase’. Thus, the PDF of the evaluated speckle phase can be obtained. The PDF of the evaluated speckle phase never becomes a uniform distribution that is the physical property of the fully developed speckle field because we cannot make a correct assumption for the unknown object phase in advance.

Figure 2 shows the PDF of the evaluated speckle phase, and its dependence on the phase error between the assumed and the actual object phases. Here, the phase error is decomposed into two terms, symmetrical $\Delta\Psi_s$ and antisymmetrical $\Delta\Psi_a$ phase terms. It can be seen clearly from Fig.2 that even fairly small phase value of $\Delta\Psi_a = 2\pi/100$ can cause a prominent non-uniform distribution. According to a precise analysis, the actual object phase can be determined from the deviation of the PDF of evaluated speckle phase from the uniform distribution of the actual speckle phase. This is the principle of the statistical interferometry. The computer simulation was carried out with the average intensity being unity. The determination error of the object phase is shown in Fig.3 as a function of the number of speckle intensity data samples N with the standard deviation of the noise component $\sigma_n = 0, 0.01, 0.1$, and 0.5 . The broken line is fitted to those values. It can be seen from Fig.3 that the determination errors of the object phase are reduced as the number of data samples N is increased, and the accuracy of $2\pi/1000$ is attainable with about $N=30000$.

3. EXPERIMENTS AND DISCUSSION

3.1 Preparation of samples and preliminary experiment

The speckle interference patterns were acquired every half a second over several seconds, and the phase changes due to the growth of the plant were analyzed according to the algorithm of statistical interferometry. Several experiments were carried out to demonstrate the validity of the present method to investigate the behaviours of plants under the various environmental conditions. We used Liriope (*Liriope muscari*) and Chinese chives (*Allium acuminatum*). The dependences of the growth speeds of plants on the strength of the light irradiance and ozone exposure were examined.

We have already established the method of the statistical interferometry through the measurements of displacements, e.g., thermal expansion of non-biological objects. However, its validity for biological investigation remains to be demonstrated. In case of such measurements, there exists random motion of the scattering particles in the tissue of the plant and this leads to degradation of the accuracy of the measurement of the plant growth. Part of the illumination beam penetrates inside the plant and dynamically scattered to produce dynamic speckle

Fig.3 Determination error of the object phase as a function of the number of data samples with the standard deviation of noise component $\sigma_n = 0, 0.05$, and 0.1 .

Fig. 4 Growth of Chinese chives. Growth rate over 5.5 sec is $1.21(\text{nm/sec mm})$

field known as biospeckles⁵. The biospeckle should be suppressed in the current measurements because it reduces the correlation of speckle patterns. To solve this problem, authors have tested various methods to cover the surface to prevent the beam from entering inside the plant. In the experiments, the surface of the leaf was covered with white wheat flour over the area to be illuminated. It was confirmed that this preparation of the plant could effectively suppress the biospeckles.

First, the growth speeds were measured for the *Allium acuminatum* at the position 1cm from the apex of the leaf. Figure 4 shows the experimental result. As is clear from the figure, the plant leaf is growing almost linearly in time over the range of 5 seconds, and the growth rate is 1.21 nm/sec·mm. Thus, it has been proved that the present method can be a powerful tool to study the growth behaviour of the plant with a high temporal resolution of the frame rate of the video system used and a high accuracy of sub-nanometer scale of the expansion. This is the great development from the conventional method in botany which is based on a dry weight. In such a traditional method, the dry weight of the plant is measured after several days or weeks. Therefore, the behaviour of the plant is known only as a result of the integration of growth over such a long term.

3.2 Dependence of growth speed on the light illumination to the plant

The condition of light illumination to the plant is also an influential factor to the plant since the carbohydrates are produced through the photosynthesis. Next, the dependence of the growth speed of *Liriope* on the light radiation was measured. The plant was illuminated by fluorescent lamps with the intensity 0 to 100,000 lux. The response of the growth on the switching of light radiation to the plant was examined. Before starting the experiment, the plant was kept under the light radiation of the intensity 50,000 lux for 2 hours. Then the growth speeds were measured for 2 hours in the dark and for the next 2 hours under the radiation of 50,000 lux. In the next two hours, the light was again turned off. Figure 5 shows the experimental results. It was found that the average growth speeds in the dark were 1.3 and 0.64 nm/sec·mm while those under the radiation of 50,000 lux was 4.9 nm/sec·mm. It can be seen that the growth speed of the plant clearly depends on the condition of light radiation. Moreover, it is remarkable that the temporal response on the radiation is fairly fast, i.e., comparable or less than the sampling interval, 10min., of the measurements.

Fig. 5 Variation of the growth speed of *Liriope* under the switching of light radiation to the plant.

3.3 Measurement of plant growth under the influence of ozone

Photo-chemical oxidant is the typical phenomenon caused by air pollution that is due to the recent development of human activity, and is giving a serious influence to animals and plants. Therefore it is worth to develop a method to estimate the stress due to pollution. Chinese chives that had grown to be approximately 20cm in leaf length after cutting back was exposed to ozone that is the main substance of photo-chemical oxidant, and the variation of the growth rate was measured.

In fig. 6, the experimental results are shown for the ozone concentrations of 0.24 ppm. Ozone concentration of 0.24 ppm is the level where photo-chemical oxidant warning is issued. It can be clearly seen that the average growth

rate before exposure, 4.2nm/sec-mm, rapidly decreases to be minus in a short period of 30min. Even after the exposure of ozone, the average growth rate is almost 0 or slightly minus, and it never recovered to its original state at least for 700min. Minus of the growth rate means the leaf started to shrink. Moreover, the most significant and interesting thing would be that the growth rate of the plant is never constant but largely fluctuating that can be seen typically for Time<0, on the contrary to our common understanding for the behaviour of plant. After the biological activity is reduced due to toxic ozone exposure, the standard deviation of the fluctuation of growth rate also becomes smaller to be 0.2nm or 13% of that in normal condition (Time<0). It was also found that the reduction in the growth rate of the fluctuation depends on the amount of the ozone exposure.

Fig. 6 Measured growth rate of Chinese chives leaf before and after exposure of 0.24ppm ozone for 3hours

4. CONCLUSION

In this study, we have applied the statistical interferometry to monitor the condition of plant, i.e., biological activity or growth aiming to monitor the influence of environmental conditions, especially for the air pollutant, ozone. In the statistical interferometry, a standard of the phase is not sought in the deterministic wavefront but the complete randomness of the fully developed speckle field. The advantage of the method is that such a stable and reliable standard of the phase can be obtained immediately by just illuminating an optically rough object commonly found around us. Moreover, because of the statistical basis of the method, improvement of the accuracy is inherently assured by taking more data samples into account.

In the experiments, it has been clearly demonstrated that the statistical interferometry has a high sensitivity of nm scale with a high temporal resolution of second scale. A nanometer scale fluctuation of the growth rate of the plant is newly revealed, and is also confirmed with other species of plant. The authors are expecting such behaviour is reflecting very fundamental aspect of biological activity. The reduction of the nanometer scale fluctuation of the growth can be a measure of the environmental stress to the plant.

REFERENCE

- 1) H. Kadono and S. Toyooka, "Statistical interferometry based on the statistics of speckle phase," Proc. of Second International Conference on Photomechanics and Speckle Metrology, SPIE, **1554A**, 718-726(1991).
- 2) H. Kadono and S. Toyooka, "Statistical interferometry based on the statistics of speckle phase," Opt. Lett., **16**, 883-885(1991).
- 3) Hirofumi Kadono, Yoshimori Bitoh and Satoru Toyooka, "Statistical interferometry based on a fully developed speckle field: an experimental demonstration with noise analysis," J. Opt. Soc. Am A, **18**, pp.1267-1274 (2001).
- 4) J. W. Goodman, in *Laser Speckle and Related Phenomena*, J. C. Dainty, ed. (Springer-Verlag, Berlin, 1975), Chap.2.
- 5) A. Oulamara, G. Tribillon and J. Duvernoy, "Biological Activity Measurement on Botanical Specimen Surfaces Using a Temporal Decorrelation Effect of Laser Speckle," J. Modern Optics, **36**, pp.165-179 (1989).