Living Plant Observation by Laser Speckle Microscopy

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Observation of a living plant was performed by laser speckle microscopy. The merit of this technique is that it is possible to easily obtain information of organisms without any special preparations, such as staining and gene transfection. In this study, a living leaf was observed for half a day at intervals of one hour. To our knowledge, this is the first report of a long-term tissue observation by the laser speckle. It was possible to visualize the fading process of the leaf cut off from the living plant. It was found that the temporal fluctuation of the speckles on the leaf completely ceased within 10 hours after cutting off the leaf from the plant body.

Key Words: Laser Speckle, Laser Microscope, Tissue Observation, Cell Observation

1. Introduction

Although observing living cells and tissues is an essential operation in the field of medicine, biology, and biotechnology, observation methods for plant cells and tissues are limited to only a few variations of techniques. Staining samples is a representative method, however, the samples need be fixed, which means the samples cannot be alive. Effective and easy observation methods for living plant cells are long-awaited.

To observe living cells, we recently constructed a laser speckle microscope, which comprised of an optical microscope, a laser source, a CCD camera and a personal computer to process the images captured by the CCD camera. This microscope can visualize activities of living cells and tissues by estimating time-varying fluctuation of the laser speckle interference patterns with a very weak laser illumination. The merit of this technique is the ease in obtaining information of organisms without any special preparations such as staining and gene transfection.[1, 2)]

In this study, observation of a living plant was performed by the laser speckle microscopy. A living leaf was observed for half a day at intervals of one hour. To our knowledge, this is the first report of a long-time tissue observation by the laser speckle.

2. Experimental

Figure 1 depicts the experimental setup. A fundamental microscope was a home-made system using C-mount components (Edmund Optics) and an objective (Nikon, CFI LWD 20×, 0.40 NA). The smallest aperture diameter inside the microscope was 8 mm in the objective. In this experiment, a laser diode (Sigma, LDU33-635-3, 635 nm, 3 mW, CW) was used as an illumination source which irradiated the samples in the horizontal direction (the incident angle of about 85 deg.) with an average power of 200 – 300 μW. The observed images were recorded into a personal computer via a CCD camera (JAI, CV-A11) controlled by commercial software (National Instruments, LabVIEW & IMAQ).

The specimen used for the investigation was the edge of a leaf of a living plant (*Muelenbeckia axillaries*), in which strong time-varying fluctuations of the laser speckle pattern were observed, and the image data was taken for half a day at intervals of one hour under a constant room temperature and humidity (26 ˚C, 50 %).

3. Results and Discussion

The images of the observed living leaf are shown in Fig. 2, in which the bright field image and the laser speckle image are put together. This leaf was not yet to be cut off from the body of plant. When the observation region in the leaf was

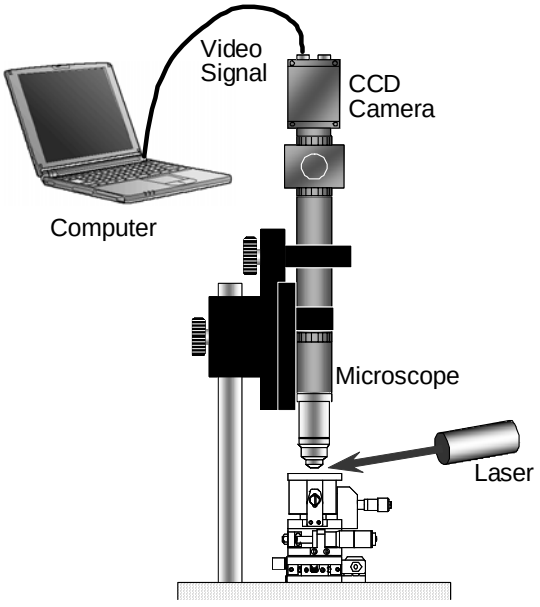
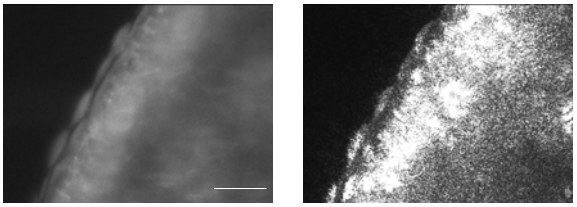


Fig. 1 Experimental setup



(a) (b)

Fig. 2 Images of a living leaf (a) for the bright field observation and (b) for the laser speckle observation. The scale bar is 50 μm.

changed, it was found that the laser speckle fluctuation on an edge of the leaf was more active than any other regions of the leaf and that the condition of the observed laser speckle on a living leaf was slightly different from that on human cells.[1-3)]

The images obtained by the laser speckle microscopy consist of static speckles and dynamic speckles which cause speckle fluctuations.[1, 2, 4)] In this experiment, the static speckle came to the front and the dynamic speckle was posteriorly-located, while the dynamic speckle was mainly observed in the case of human cells.[5)] This may be explained by the different cellular structure between plants and human or animals. Plant cells have cell walls that do not exist in human (animals) cells and are constitutively rigid and stationary. In addition, a cuticle membrane including wax components covers and protects the surface of plant cells. As a result, the static speckle component caused by cellular structures such as cell walls and cell membranes became remarkable, while the dynamic speckle component, which reflects cellular activity and which is caused by movements of intracellular materials, appeared to be relatively suppressed.

The speckle fluctuation had been processed with a computer program (MathWorks, MATLAB) utilizing fast Fourier transformation (FFT) in the case of human cell observations[1,2)]; however, it was necessary to prepare a special algorithm to evaluate the laser speckle fluctuation on the leaf because of the behaviour difference of the laser speckle between leaves and human cells. The scheme as shown in Fig. 3, which removes the static speckle effect, was based on the difference method.[6)] This method visualizes the intensity (brightness) difference in each pixel between one still frame and the next frame. This method made it possible to clarify very small laser speckle fluctuation on a leaf, and the process speed was faster than that by the FFT method, although the frequency spectra of the speckle fluctuation could not be

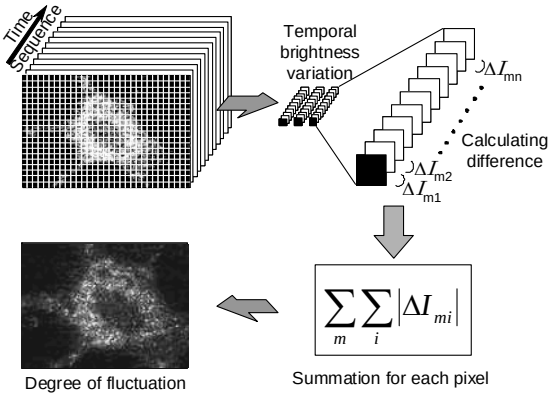


Fig. 3 The scheme for estimation of the laser speckle fluctuation using the difference method.

obtained.

Figure 4 is estimated results of a long-term observation for a leaf fading process visualized by the laser speckle microscopy, in which this difference method was used as the analyzing scheme. In this experiment, the leaf was observed for half a day at intervals of one hour. The speckle fluctuation on the leaf completely ceased within 10 hours after cutting off the leaf from the plant body. Although in the early study of bio-speckle, the speckle observation on plants (fruits and algae) was reported so far,[7)] to our knowledge, this long-term speckle observation of a plant is the first report. In Fig. 4, a white colour indicates most active speckle fluctuations and black colour shows completely stationary speckles. A dark area at upper-left in each image in Fig. 4 represents the background (only a slide glass without specimen). As seen in Fig. 4, area of white colour gradually decreased as time elapsed and after 9 h, most of the imaged area became covered with black regions. This situation well agrees with the time-varying speckle fluctuation observed with naked eyes, and it suggests the effectiveness of the processing technique. In fact, the observed leaf slowly faded as time goes by, therefore, it is reasonable to believe that the series of the processed images in Fig. 4 show the death process of the leaf.

In Fig. 5, the calculated quantity corresponding to the averaged speckle fluctuation level is plotted against the elapsed time. This fluctuation level was obtained by calculating the average intensity (brightness) of the processed

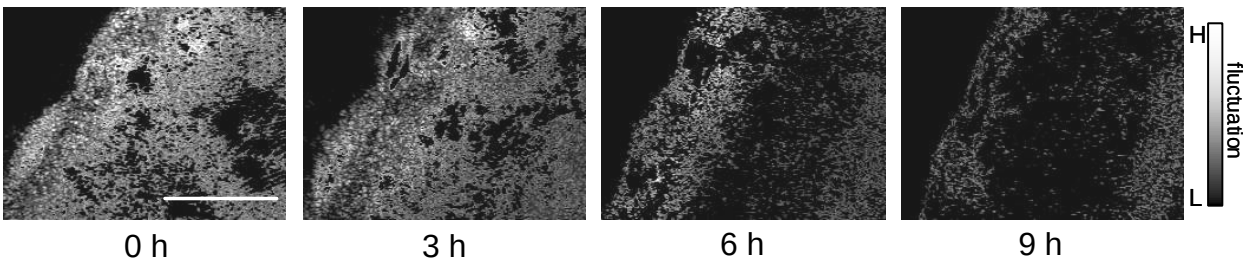


Fig. 4 Long-time variation of the laser speckle fluctuation on the living leaf. "0h" means just before being cut from the plant, and "3h", "6h", and "9h" are elapsed times since the leaf was cut off from the plant body. The scale bar is 50 μm.

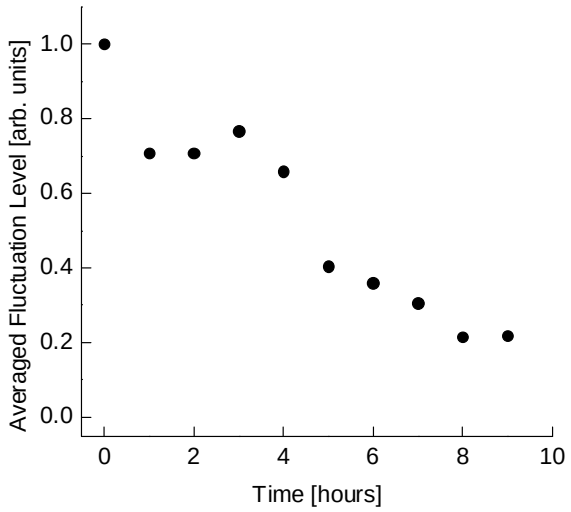


Fig. 5 Long-time variation of the averaged laser speckle fluctuation level (the averaged brightness of the processed images like as Fig. 4) on the living leaf.

image such as 4-images in Fig. 4. As white colour indicates the most dynamic movements of the speckle fluctuation, the averaged brightness of the individual processed images, like those shown images in Fig. 4, can be consider as the averaged fluctuation-activity of the speckle image at the observed moment. It is found from Fig. 5 that immediately after cutting off the leaf from the plant body, the leaf-activity drastically decreased by 30% and it gradually subsided over time. Since this tendency is consistent with the time-varying fluctuation of the laser speckle movies recorded by the laser speckle microscope, the fluctuation level calculated here could be considered as a highly useful index parameter.

Although it is not still clear what kind of phenomena or material movements inside the leaf are responsible for such observation of speckle fluctuations, a number of possible causes can be cited. When a region of the leaf vein was focused for close observation, some very fast flow like water often could result in the time-varying speckle fluctuation. This fact suggests that the speckle fluctuation shown in Fig. 4 might reflect water flows inside the leaf. However, the phenomenon of the water flow cannot sufficiently explain the fact that the most dynamic fluctuations were observed on the edge region of the leaf as described above. Considering the case of human cells observations, it is reasonable to assume that movements of some intracellular biomaterials affect the speckle fluctuation as the fluctuation causes [1 - 3, 8]. Further detailed investigations about this point are necessary in order to ascertain the underlying mechanisms.

In conclusion, observation of the living plant was performed by laser speckle microscopy. The living leaf was observed for half a day at intervals of one hour. It was possible to visualize the fading process of the leaf without any special preparations. This experiment suggests that the laser speckle microscopy is a potential tool for living-plant observations.

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