Environmental Pollution Assessment with Indicator Plant Under Ozone Gas Atmosphere by Using OCT

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Abstract: Measuring plants' sensitivity to environmental stresses can help us understand the environmental and ecological conditions in the area. Optical Coherence Tomography (OCT) can visualize and evaluate the internal plant structure quantitatively. In this study, as a preliminary step to assess the atmospheric environment by field measurements of plants using OCT, the influence of the ozone gas and the effect of the plant-clearing agent in OCT measurement were evaluated. The plant-clearing agent makes the internal refractive index uniform and allows evaluation of the extinction coefficient from the leaf's full cross-section image. The results showed an increase in the extinction coefficient and its palisade thickness. The extinction coefficient significantly changed between before and after exposure to ozone gas to 16.9 ± 6.2 [/mm] from 12.6 ± 3.42 [/mm]. This result indicates that OCT can measure plant responses to environmental changes quantitatively. Field measurement of plants by OCT will allow environmental assessment anywhere in a short time.

1 INTRODUCTION

In recent years, toxic substances in the air have become high enough to affect plants and animals in some urban areas (Masui et al., 2021, Graham et al., 1998). One method of environmental assessment is to use "indicator plants" that are sensitive to changes in the atmospheric environment. This method does not need expensive equipment. In addition, indicator plants can be evaluated by visual process or by using the conventional technique. If the atmosphere becomes dry, the chlorophyll content in plants' leaves decreases. Spectroscopic observation can observe those decreases (Kwartiningsih et al., 2021). However, the reduction of chlorophyll can also be caused by nutrient deficiencies and insect damage. Spectroscopic observations can also be used to observe plants' responses that lead to pigment changes, biochemical changes, and inhibition of photosynthesis. Because of the simultaneous occurrence of different plant stressors, it is not easy to elucidate the causal stress.

If a plant experiences environmental stresses, the effect of this stress is often found in the changes in the plant's internal structure. For this reason, close-range remote sensing for observing plant growth is a practical approach to evaluating environmental stressors by measuring plant conditions. Optical Coherence Tomography (OCT) is a remote sensing technique that uses near-infrared light to visualize the internal structures of living organisms. It has the advantage over other methods, such as MRI, X-ray, etc., in obtaining internal structures by being inexpensive, compact, and can be used in the field (Wijesinghe et al., 2017, Lee et al., 2019). This research discusses the feasibility of in-situ observation of the atmospheric environmental stress on plants by measuring the change in leaves' internal structure.

One problem with OCT is that the measurement depth is limited due to light scattering due to the non-uniformity of the refractive index of a plant leaf's tissue (Wit et al., 2020). To match the internal refractive index, we used a plant-clearing agent with a refractive index close to the plant tissue that can penetrate inside the plant (Villani et al., 2013). We discussed the advantages of tissue clearing in field observations with OCT.

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We focused on ozone gas as environmental stress on plants. Ozone gas is relatively high near industrial and urban areas. Since high ozone concentrations can affect plants, quantifying the effect of ozone gas on plants is an essential aspect that this work wants to establish. In this work, we exposed white clovers, the ozone indicator plant, under a high concentration of ozone gas and measured changes in the leaf's internal structure by using our original OCT. These results can be used to infer ozone concentration in a measurement area.

This work aims to evaluate the plant growing environment by using OCT in orchards or polluted areas. The OCT that was developed in our laboratory can evaluate quantitatively by only point measurement with the discussion of their optical properties. The proposed method has the advantage of easy environmental assessment anywhere.

2 METHOD

The OCT system used in this study is developed for plant measurement in our laboratory, and its configuration is shown in Fig.1(a). Fig.1(b) shows the optical probe and its' measurement scene. The size of this OCT is shown in Fig.1(c), and it is small enough to be easily taken it outside and runs on a DC battery. This OCT, a TD-OCT, incorporates a rotation mechanism in the reference optical path, and the optical path length changes linearly with time. This OCT is constructed to evaluate changes in the plant's internal structure by point measurement. Leaves are quantitatively evaluated by acquiring A-scans in field measurements. We select the wavelength of the light source to be 1310 nm, which has a low absorbance for chlorophyll and a local minimum absorbance for water. The optical probe was designed to be small. The probe is small enough to be positioned anywhere near the leaf and at any angle during field measurement. Table 1 shows the specification of the OCT system. A-scan signals are acquired at 25 Hz, and each A-scan is the average of 16 measurements to reduce noises.

In the OCT measurement with a plant-clearing agent, the leaves were placed between two acrylic plates with a window in the measurement area (Fig. 1b). This method needs to measure the same position of the leaf before and after the transparency process. For this reason, the plant-clearing agent is filled into the window, with the leaf in between two acrylic plates. We used Visikol (Visiko Inc.) as the plant-clearing agent, which has a refractive index equivalent to plant tissue and makes the internal refractive index uniform as it penetrates the interior of the leaf. A uniform refractive index reduces internal scattering and allows light to reach deeper inside the plant tissue.

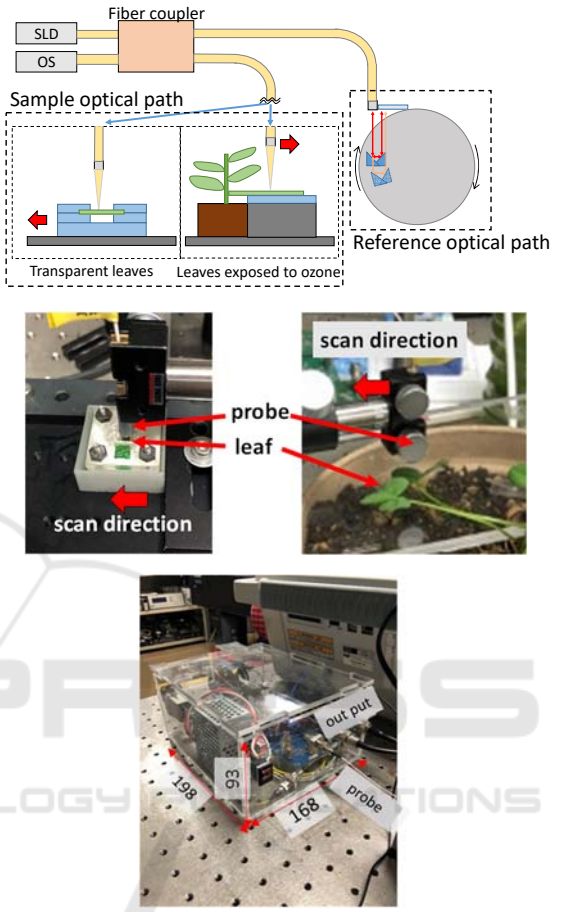
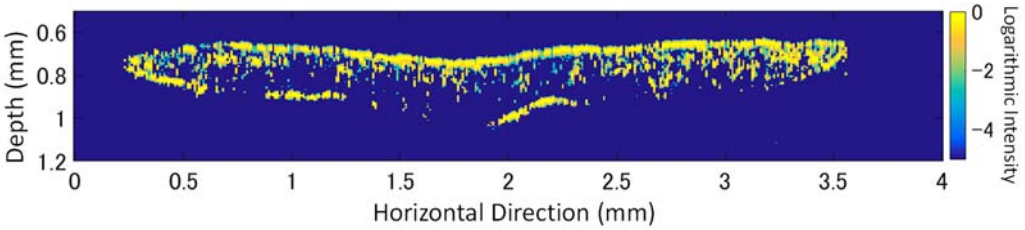


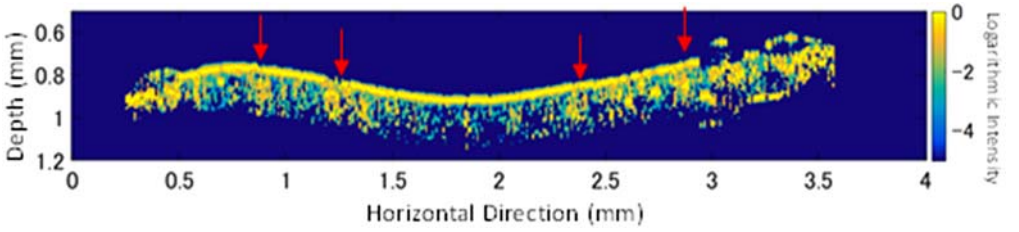
Figure 1: a) The configuration of the OCT system; b) scanning of mounted transparent leaf (left) and probing of leaves exposed to ozone (right); c) control box of the OCT system.

Table 1: Specification.

Item	specification
Center Wavelength	1310 nm
SLD Output	15 μW
FWHM	53 nm
Axial Resolution	14.2 μ m
Lateral Resolution	10 μm
A-scan Rate	25 Hz
Average Times	16
Focal Length	5.12 mm
NA	0.13
Beam Diameter	0.65 mm
OCT Size	198 × 168 × 98 mm
Probe Size	ϕ 6 mm×9 mm



(a): Before transparency process.



(b): After transparency process.

Figure 2: Transparency of Dracena leaves.

In the case of white clover, measurements are made without cutting the leaves and keeping the plants in pots so that the temporal changes of the leaf's internal structure due to the effect of ozone gas can be measured with the same leaf. White clover was grown in an incubator at 20°C, and the ozone concentration in the incubator was approximately 0.21 ppm. In addition, the blue and red light of the cold-cathode lamp is turned on 15 hours in a day. Leaf measurements were taken every 12 hours after exposure to ozone gas.

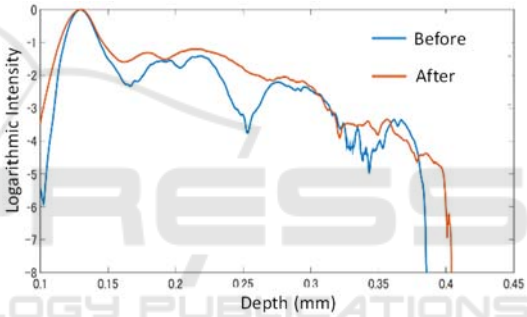


Figure 3: Averaged intensity change with depth in Figure 2.

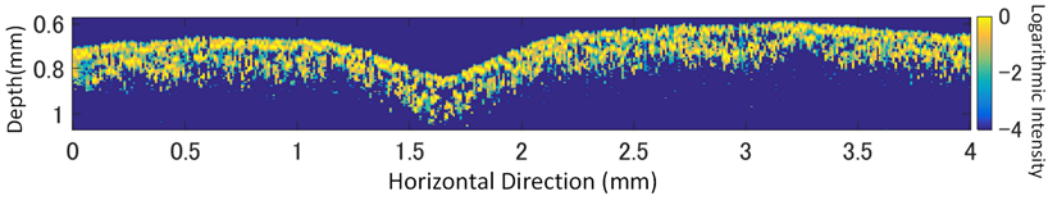
3 RESULTS

3.1 Transparency Process of Dracaena Leaves

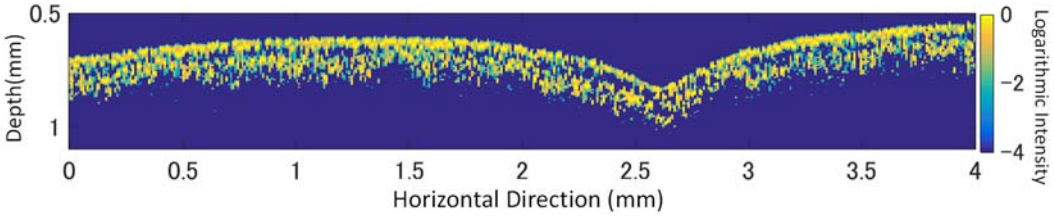
Figures 2 (a) and (b) show the results of Dracaena leaves before and after the transparency process with Visikol, respectively. The x-axis in the figure represents the horizontal point (B-scan direction), and the y-axis is the depth direction (A-scan). OCT light is illuminated from the top of the leaf's surface. The intensity of interference light is indicated by the color bar. Furthermore, the intensity of interference light is obtained by subtracting the background light, and performing focal length correction, distance squared correction, and logarithmic transformation from the obtained original OCT signals.

The result before the transparency process is shown in Fig.2(a). The adaxial surface is visualized and a part of the abaxial surface is visualized, too. In the leaf's interior, the signal from the layer near the adaxial surface is partially visualized, while the layer on the abaxial surface is hardly visualized. In the result after the transparency process with Visikol shown in Fig. 2(b), the entire adaxial surface is visualized, but the abaxial surface is not. In the leaf's interior, the signal is obtained throughout the leaf's full cross-section image. The area indicated by the red arrow in Fig. 2(b) has the signal of the leaf veins, and the signal is stronger than in the other areas. The signal of the abaxial surface is only obtained at the right end part, and the signal disappears to the left of 3.0 mm of the x-axis. Similarly, the signal also disappears at both ends of the adaxial surface. This disappearance of the abaxial surface is caused by delaminating the surface due to the transparency process.

The adaxial surface position in Fig. 2 is aligned at the same depth, and then the intensity changes with depth direction are averaged in the horizontal direction. In this case, both ends of the leaf and the



(a): Before exposure to the ozone gas.



(b): After exposure to the ozone gas.

Figure 4: White Clover under ozone gas.

veins are excluded from the averaging procedure. Figure 3 shows these results. The y-axis shows the average of the logarithmic intensity, and the x-axis shows the depth. The blue line shows the results before the transparency process, and the orange line shows the results after the transparency process with Visikol. Before the transparency process, the peak intensities in the adaxial and abaxial surfaces can be seen. After the transparency process, the abaxial surface signal disappears. Inside peaks are obscure, and its attenuation is monotonous.

The plant-clearing agent reduces the internal refractive index differences. Since the plant-clearing agent increases the light transmission of the leaves' interior, the veins' location and thickness of the layer become clear. In Fig.3, the transparency process made the constant attenuation. Therefore, the extinction coefficient can evaluate from the leaf's full cross-section signal. The use of the plnat-clearing agent increases the OCT signal near the abaxial axis side of the leaf. It can evaluate the change of light transmission due to disease or environmental stress in the field measurement.

3.2 Measurement of White Clover Under Ozone Gas Atmosphere

Figure 4(a) shows the result before the white clover was exposed to ozone gas, and Figure 4(b) shows the result after 12 hours under ozone gas exposure. In the same way as Fig.2, these figures show the results of modified intensity by subtracting the background light, applying focal length correction, distance squared correction, and logarithmic transformation from the obtained OCT signals. The entire adaxial surface was visualized before and after exposure to ozone gas, and the abaxial surface was not visualized. The interface between the epidermis layer and the palisade tissue became clearer after exposure to ozone gas.

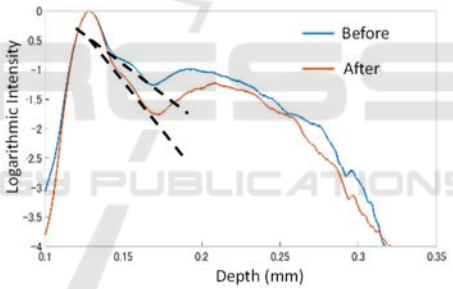


Figure 5: Intensity change with depth in Fig 4.

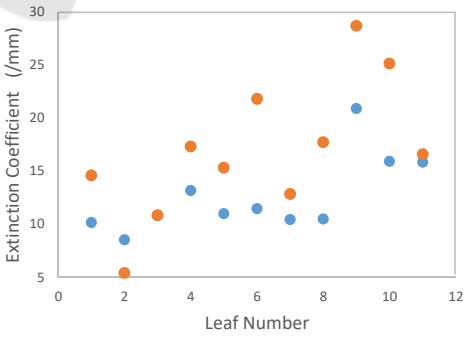


Figure 6: Extinction coefficient of multiple leaves in palisade tissue.

Similar to Fig. 3, the surface in Fig. 4 is aligned and the A-lines are averaged, except for the position of the veins. Figure 5 shows these A-line average results. The blue curve in the figure shows the result

before exposure to ozone gas, and the orange curve shows the results after 12-hour exposure to ozone gas. The interface of the palisade and spongy tissues (at the 0.17 mm depth position in Fig.5) is almost unchanged before and after exposure to ozone gas. However, the attenuation of the signal in the palisade tissue is increased by exposure to ozone gas. The signal below the palisade tissue is unchanged. The effect of ozone gas on plant leaves appears in the palisade tissue (Thomson et al., 1966, Hartikainen et al., 2020).

We measured 11 white clover leaves exposed to ozone gas for 12 hours. Figure 6 shows extinction coefficients in the palisade tissue. The extinction coefficient is the slope of the black dotted line in Fig. 5. The x-axis in Fig. 6 indicates the leaf number, and the y-axis shows the extinction coefficient. The blue and orange circles in the figure represent the measurement before and after 12 hours of exposure to ozone gas. The two vertically aligned blue and orange circles are the results of the same leaf.

The results show an increase in the extinction coefficient of the palisade tissue in most leaves. The mean value of the extinction coefficients before and after exposure to ozone gas is 12.6 ± 3.42 [/mm] and 16.9 ± 6.2 [/mm], respectively. After exposure to ozone gas, the extinction coefficient increased, and the standard deviation is larger. A one-tailed *t*-test was performed on this result as $p = 0.003$ ($p<0.05$). These findings confirm that ozone destroys the palisade tissue, and these changes can be evaluated quantitatively from OCT signals.

4 CONCLUSIONS

In this study, we investigate the effect of the plnat-clearing agent on the OCT images and the ozone gas on the plant tissue to assess the environmental contamination of ozone by OCT for the purpose of future field measurements. The transparency process increased and homogenized the internal OCT signals from leaf measurements. The ozone gas affects the epidermis tissue of white clover and significantly increases the extinction coefficient obtained by OCT in the palisade tissue.

Soaking the leaves in Visikol, the signal only visible near the epidermis layer became uniformly visible from the adaxial surface to the abaxial surface. The position of the veins, which had originally been unclear, could be confirmed, too. In addition, the extinction coefficient inside the leaf becomes uniform due to the decrease of the refractive index difference by the penetration of the plant-clearing agent. It makes it possible to evaluate the change of extinction coefficient over the leaf's full cross-sectional signal. The transparency process can confirm the disease or environmental stress, which changes the transmittance of light. Since the plant-clearing agent can make the leaf's full cross-sectional signal clear, diseases or environmental stresses affecting the whole region of interest or region of the leaf where we don't know are affected can be detected by the proposed method. Because this method can detect by point measurement, OCT can quantitatively and speedily assess environmental conditions.

When plant leaves are exposed to high concentrations of ozone gas, the palisade tissues are destroyed. It has been difficult to clearly distinguish these changes from mere OCT images. By averaging each A-scan and comparing before and after exposure to ozone gas, a clear difference in the extinction coefficient in the palisade tissue appeared. On the other hand, the spongy tissue was almost the same before and after the ozone gas exposure. In addition, we measured multiple leaves, which confirmed that palisade's extinction coefficients predominantly changed before and after exposure to the ozone gas by *t*-test.

In the leaf measurements with transparency process and exposure to ozone, the changes can be confirmed by A-scan, as shown in Figs. 3 and 5. Thus, it is possible to confirm the effect of ozone when white clover leaves from areas with high and low concentrations of ozone are observed using point measurements by this OCT method. Since this result shows that our portable OCT can detect the change in the leaf's interior, OCT can estimate the environmental conditions by measuring indicator plants. In addition, the OCT with additional functions can observe changes in the internal structure of plants, and more accurate environmental evaluation can be performed. For example, polarized OCT, which can observe separately p-polarization and s-polarization, can capture changes in the internal polarization state of plants, and b-OCT, which visualizes the speckle variation with time, can evaluate plant activity (Silva et al., 2021). If this method is established, this portable OCT can be a useful instrument to assess the identification or predict comprehensive environmental stress.

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