Microstructure, Extinction Coefficient, and Chlorophyll Content of Philippine Bamboo Leaves by a Portable TD-OCT Scanner

Jumar Cadondon[1,2,3][a], Edgar Vallar[1][b], Maria Cecilia Galvez[1][c] and Tatsuo Shiina[3][d]

[1]*Department of Physics, College of Science, De La Salle University, 1004 Taft Avenue, Manila 0922, Philippines*

[2]*Division of Physical Sciences and Mathematics, College of Arts and Sciences-Miagao Campus, Miagao 5023, Iloilo, Philippines*

[3]*Graduate School of Engineering, Chiba University, Yayoi-cho, Chiba 263-8522, Japan*

Keywords: Philippine Bamboo, Extinction Coefficient, Microstructure, Chlorophyll, TD-OCT.

Abstract: Bamboo is one of the most utilized non-timber forest products in the Philippines. Common bamboo leaf infections are caused by sunlight, and nutrient deficiency. In this study, we have developed a portable time domain-optical coherence tomography (TD-OCT) to study in vivo leaf microstructure changes in Philippine bamboo (*Bambusa spinosa*). TD-OCT analysis shows unique features among different layers of the leaves specifically on the epidermis and palisade layers when the unhealthy part is compared to the healthy part. Extinction coefficient from the A-scan analysis showed significant difference from unhealthy part (1.03 ± 0.20 mm^{-1}, N =12, $p<0.05$) and healthy part (0.72 ± 0.27 mm^{-1}, N =12, $p<0.05$). In addition, RGB data was compared for both unhealthy and healthy part of the bamboo leaves. A red shift is observed from the unhealthy part of the leaves. It is also observed by a decrease of 60% in chlorophyll-a content of the unhealthy part as compared to the healthy part. Same inverse correlation is also observed when the extinction coefficient is compared with the chlorophyll content.

1 INTRODUCTION

Bamboo is a diverse group of perennials with emerging use in food, handicrafts, chemical products, and building materials (Cheng et al., 2023). The microstructure of leaves is crucial in the overall development of plants. Leaf growth depends on its photosynthetic ability and leaf phenology. Visual inspection shows unhealthy and healthy structure within the leaves. Furthermore, the loss of the photosynthetic ability of the plant is due to environmental factors such as temperature, humidity, nutrition, and oxidative stress (Liu et al., 2022). Several methods have been introduced in leaf growth dynamics and early detection of leaf diseases. Such inspection are subjective, inefficient, time-consuming methods in early detection of leaf diseases. Understanding such relation between chlorophyll concentration and its microstructures has not been further studied.

Traditional methods using biomedical techniques have been commonly used to study photosyntheitc ability in plants such as chlrophyll content, protein, lipids, and fats (Cao et al., 2013). However, these studies mostly focus on the cell components. In vivo technique using microstructural patterns have been a growing research on the leaf plant development. Changes in the leaf microstructures are mostly associated with its photosynthetic ability. Imaging techniques such as scanning electron microscopy (SEM), transmission electron microscopy (TEM) (Wang et al., 2014; Yao et al., 2017), confocal and fluorescence microscopy (Zhao et al., 2016) and spectroscopic methods (Butler et al., 2015; Ivanova and Singh, 2003) can provided cellular, molecular data, however, are limited by penetration depth which requires plant sectioning.

Optical coherence tomography (OCT), is a non-invasive technique that can provide high-speed cross-

[a] https://orcid.org/0000-0002-3933-0598
[b] https://orcid.org/0000-0001-8236-7102
[c] https://orcid.org/0000-0001-5505-1778
[d] https://orcid.org/0000-0001-9292-4523

Cadondon, J., Vallar, E., Galvez, M. C. and Shiina, T.
Microstructure, Extinction Coefficient, and Chlorophyll Content of Philippine Bamboo Leaves by a Portable TD-OCT Scanner.
DOI: 10.5220/0013244900003902

In *Proceedings of the 13th International Conference on Photonics, Optics and Laser Technology (PHOTOPTICS 2025)*, pages 114-119
ISBN: 978-989-758-736-8; ISSN: 2184-4364

sectional imaging with micrometer resolution in highly scattering samples. OCT has been widely used for retinal imaging, dermatology, forensic studies (Popescu, 2014; Meglinski et al., 2010). There are increasing trends in using OCT in biomedical applications in plant biology and agriculture (Goto et al., 2023). In this study, we have developed a time domain- OCT (TD-OCT) that shows deeper penetration in leaves. In this study, TD-OCT has been used to obtain cross-sectional images of *Bambusa* sp. leaves to illustrate microstructural differences in healthy and unhealthy part due to environmental factors. Moreover, the extinction coefficient measured from A-scan analysis was compared for both healthy and unhealthy parts of the bamboo leaves. The chlorophyll-a content was also studied using absorbance spectroscopy. Correlation between the exiction coefficient and chlrolophyll-a content has been observed using the developed TD-OCT. This is the first reported study on the use of TD-OCT imaging in elucidating microstructural changes in leaves which can be furthered explored for agricultural growth and development.

2 TD-OCT DEVELOPMENT

Our TD-OCT is based on the Michelson interferometer as shown in Fig 1. Our OCT has dimensions of 10 in. by 8 in. 5 in., which can be easily used for in-situ plant monitoring. A rotating retroreflector is designed using a reference arm (RA) instead of moving in translation motion. It has more scanning range which can be easily adjusted by changing the retroreflector's radius. A 1310 nm SLD (Anritsu Co. Ltd., Kanagawa, Japan) with a spectral width of 56 nm and average axial resolution of 14.2 µm in air was used (Shiina et al., 2003). It is constructed to evaluate the microstructural changes of the plant by acquiring A-scans as point measurement. Table 1 shows the specifications of the developed TD-OCT (Galvez et al., 2023). In this system, the probe is designed to be small and can be position easily on the surface of the leaves.

It includes super luminescent diode (SLD), photodiode (PD), signal processing circuit board (SPCB), oscilloscope (Osc), personal computer (PC), beam splitter (BS), reference arm (RA), probe (PR), sample (SP), and fiber coupler assembly (FCA).

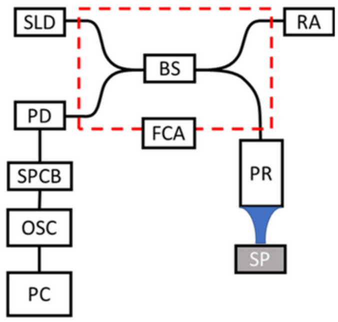


Figure 1: The schematic diagram of the TD-OCT system.

Table 1: Specifications of the TD-OCT system.

Specification	Value
Center wavelength	1310 nm
Spectral width	56 nm
Axial resolution	14.2 µm
Lateral resolution, spot size	6 µm
Numerical aperture	0.14
Scanning rate	25 scans/s
Scanning depth in air	12-14 mm

2.1 Bamboo Collection

Bamboo has disadvantages like pest and fungal susceptibility due to its small morphological structure. It has also low survival rate for micropropagation. On the other hand, growing bamboo has the ability to mitigate flood and soil erosion (DOST PCAARD, n.d.). The OCT measurement was conducted using Philippine bamboo collected from the Philippines' Department of Agriculture. Twenty-five (25) bamboo leaves were collected based on visual inspection showing both healthy and unhealthy part (Fig. 2).

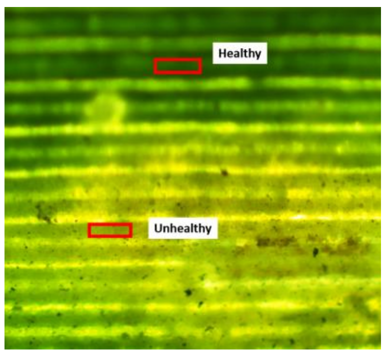


Figure 2: The bamboo leaf (top view) collected with the healthy and unhealthy part.

2.2 Microstructural Imaging, and Extinction Coefficient

In this work, all bamboo leaves were imaged on adaxial surface. The photographs (Fig. 2) emphasize the topographical and color changes. Leaves are multilayered structures which varies in absorption coefficients in different layers. Hence, A-scan and B-scan analysis was performed to obtain a detailed microstructural information from the TD-OCT images. A-scan is defined for depth scan which is also related to longitudinal scan; while B-scan is referred to the transverse sections. The thickness between two layers of a leaf can be defined between corresponding distance between the A-scan profile. With the successive A-scan analysis, a 2D-cross sectional image is created, called the B-scan.

Using the microstructural information collected, these can be quantified in terms of intensity by measuring the extinction coefficient. The extinction coefficient is an intrinsic optical property of tissues that is highly correlated with the TD-OCT signals. It is defined as the amount of light scatter and absorption per unit distance as light travels into the plant tissue.

2.3 RGB Image and Chlorophyll-a Measurement

An Olympus microscope with a high-resolution camera attached was used to analyse the adaxial information (Fig. 2) and RGB images. To measure specific frequency distribution of the RGB intensity, a region of interest (ROI) was chosen. This is also designated as the region where OCT imaging was conducted. In Fig. 2, the red rectangle represents the ROI. The difference in the RGB frequency was recorded for both healthy and unhealthy part of the bamboo leaves.

Microstructural and morphological changes are accompanied by the changes in the chlorophyll content of the leaves. Thus, we also measured the chlorophyll-a concentration by measuring the optical density (OD) of the leaves at 680 nm (Cadondon et al., 2023) and the estimated chlorophyll-a concentration based on Sartory and Grobbelaar (1984) as shown below.

$$\mathrm{Chl}-\mathrm{a}=\frac{[26.73(665\mathrm{a}-663\mathrm{b})]\mathrm{EF}}{\mathrm{VL}} \quad (1)$$

A detailed methodology is presented previously (Cadondon et al., 2022). The optical density was measured at 750 nm, 663 nm, and 630 nm wavelengths. In the equation above, 665a is the turbidity corrected absorbance at 665 nm, and 663b is the turbidity corrected absorbance at 663 nm after acidification, F is the dilution factor, E is the volume of the solved used for the extraction (mL), V is the volume of the filtered sample (mL), and L is the path length (cm). Unsaturated samples are given with a dilution factor of 1. On the other hand, dilution factor of saturated samples is dependent on the number of times the samples are diluted. In this case, the volume of the solvent also increases.

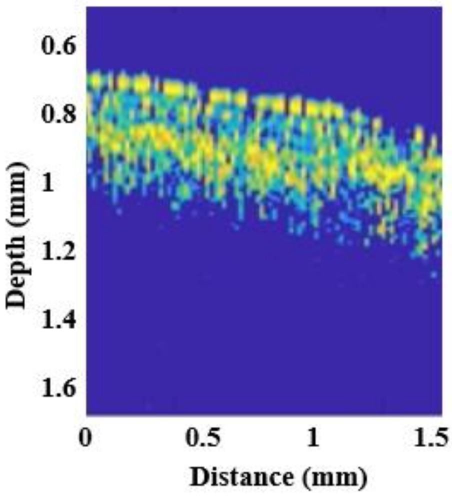
2.4 Statistical Treatments

Using Pearson's R correlation, the estimated chlorophyll-a concentrations of the bamboo leaves were correlated with the extinction coefficient measured from the OCT signals.

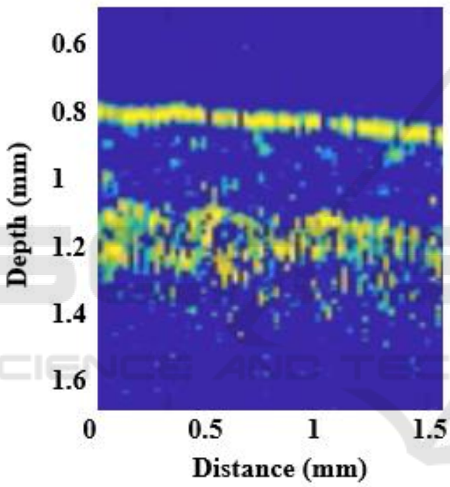
3 MICROSTRUCTURE ANALYSIS OF BAMBOO LEAVES

Figure 3 (a) and (b) shows the microstructural changes of the bamboo leaves on OCT images. Corrections were made by subtracting the background light, focal length correction, distance squared correction, and logarithmic analysis to obtained the B-scan signals. The broadening of the second order peak in the unhealthy part of the leaves is highly observed. This implies that the upper dermis and palisade layers merged to form a thick layer. The increase in the thickness and the changes in the epidermis and palisade layers can be used to explain the environmental factors affecting the color change in the bamboo leaves. Hence, it is very useful to identify microstructural changes on the leaves using OCT images to further understand its morphological changes.

This can be further verified by determining the frequency distribution of the RGB color in the leaves. Fig. 4 (a) and (b) provides the intensity-frequency distribution of the RGB image of the healthy and unhealthy part of the bamboo leaves. The x-axis is the intensity and the y-axis is the frequency distribution of the color.



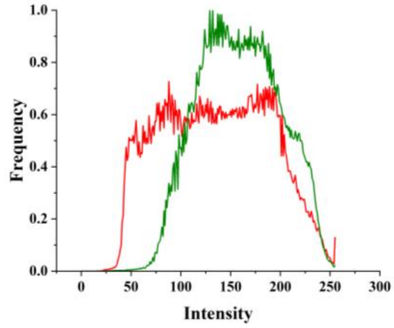
(a) Healthy part



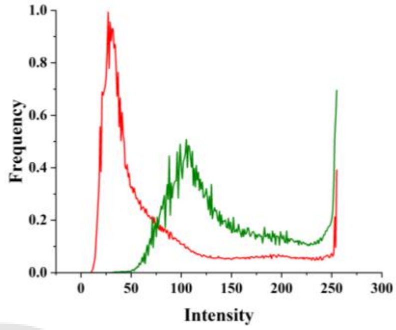
(b) Unhealthy part

Figure 3: The OCT images of the bamboo leaves collected with the healthy and unhealthy part.

The same intensity and frequency distribution is observed in a healthy part of the bamboo leaves as shown in Fig. 4(a). A decrease in the intensity profile is observed in the unhealthy part of the bamboo leaves. A shift in the red profile is also observed (Fig 4(b) (Li et al., 2012). This means that the absorption of the bamboo leaves changes as the microstructure changes (Zhang et al., 2022).



(a) Healthy



(b) Unhealthy

Figure 4: Normalized Intensity-Frequency profile of the RGB of healthy and unhealthy part of the bamboo leaves.

4 EXTINCTION COEFFICIENT, AND CHLOROPHYLL CONTENT

As discussed, extinction coefficient is important to quantitatively measure the microstructural changes in terms of intensity. Figure 5 shows the extinction coefficient estimated by OCT images. From the A-scan measured, a lineal fitting model was structured to obtain the slope of the extinction coefficient of each. The calculated mean extinction coefficients ± standard deviation for healthy and unhealthy part are: 0.72 ± 0.27 mm^{-1}, and 1.03 ± 0.23 mm^{-1}, respectively. Our results statistically show that lower extinction coefficient is observed for the healthy part as compared to the unhealthy part. This explains the broadening of first layer and the second layer with the merging of the epidermis and the palisade layer (Anna et al., 2018). The microstructural changes in the upper dermis provides information on the adapting ability to environmental factors.

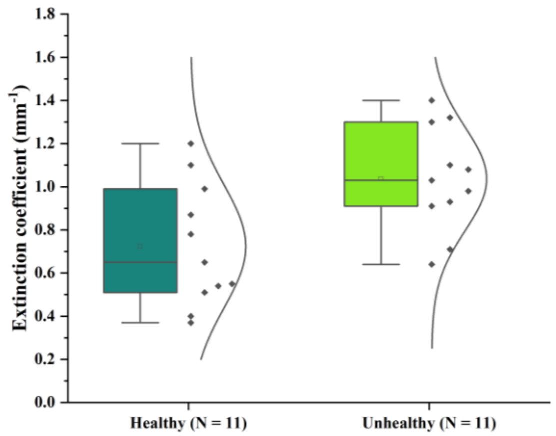


Figure 5: Mean extinction coefficient of the bamboo leaves.

To confirm the changes in the intensity and frequency distribution from the RGB data along with the morphological and microstructural changes in the bamboo leaves, the chlorophyll-a content is measured (Fig. 6). A significant decreased by 60 % was observed from the unhealthy part of the bamboo leaves when compared with the healthy part. These findings show that the chlorophyll-a content varies on the different parts of the leaves. This also confirms that such decrease in affected by environmental factors that lowers the ability of the plant to produce its own chlorophyll.

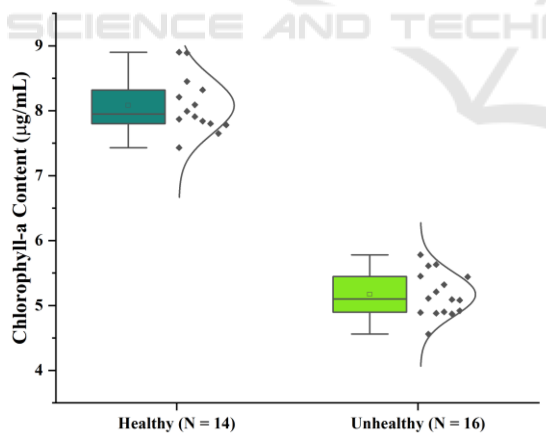


Figure 6: Mean chlorophyll-a content of the bamboo leaves.

With the significant change, it can be observed that there is an inverse correlation between the extinction coefficient of bamboo leaves based on the OCT image and the chlorophyll-a content (Inskeep and Bloom, 1985). Visual inspection through microscopy and cell analysis are commonly used in understanding chlorophyll-a distribution in plants. In this study, we were able to provide a different approach in estimating chlorophyll-a distribution using the estimated extinction coefficient from the TD-OCT images. Our TD-OCT helps investigate in the senescence of leaves without destroying the structure of the plant.

5 CONCLUSIONS AND FUTURE PLANS

We studied the microstructural changes of the healthy and unhealthy part of the bamboo leaves using the OCT images. Environmental factors such as sunlight, nutrients, and oxygen deficiencies affect the morphological structures and the ability of the leaves to produce chlorophyll. A significant change in the extinction coefficient of the unhealthy part as compared to the healthy part. This means that the penetration of the light is possible due to the change in the color. This is verified by measuring the RGB data from the same region of interest for both parts. It can be observed that the intensity and frequency distribution of red and green data are similar from the healthy leaves; while a significant shift in the red color is observed in the unhealthy part.

To understand the color changes, the chlorophyll-a content is measured. A 60% decreased was observed in the chlorophyll-a content from the unhealthy to the healthy part of the bamboo leaves. This supports the microstructural changes observed by the OCT images. Overall, an inverse correlation was observed between the extinction coefficient and the chlorophyll-a content of the bamboo leaves. With the on-going development of the portable TD-OCT scanner, we plan to extend the understanding microstructural changes associated with the senescence of leaves using our system.

ACKNOWLEDGEMENTS

J. Cadondon acknowledges support through the DOST ASTHRDP and Enrichment Program. This research was funded by the Commission on Higher Education (CHED) of the Philippine Government for the project entitled “Development of a Portable Optical Coherence Tomography System for the Evaluation of Human Skin Analogues”.

REFERENCES

Anna, T., Chakraborty, S., Cheng, C-Y., Srivastava, V., Chiou, A., Kuo, W-C. (2019). Elucidation of microstructural changes in leaves during senescence using spectral domain optical coherence tomography. *Scientific Reports* 9, 1167.

Butler, H.J., McAinsh, M.R., Adams, S., Marti, F.L. (2015). Application of vibrational spectroscopy techniques to non-destructively monitr plant health and development. *Analytical Methods* 7, 4059-4070.

Cadondon, J., Lesidan, J.R., Bulan, J., Vallar, E., Shiina, T., Galvez, M.C. (2023). Algal Organic Matter Fluorescence Analysis of *Chlorella* sp. for Biomass Estimation. *Engineering Proceedings* 58(1), 80.

Cadondon, J.G., Ong, P.M. B., Vallar, E.A., Shiina, T., Galvez, M.C.D. (2022). Chlorophyll-a pigment measurement of spirulina in algal growth monitoring using portable pulsed LED fluorescence lidar system. *Sensors* 22(8), 2940.

Cao, J. et al. (2016). Understanding studies on the natural leaf senescence of *Cinnamomum camphora. Scanning* 35, 336-343.

Cheng, Y., Wan, S., Yao, S., et al. (2023). Bamboo leaf: A review of traditional medicinal property, phytochemistry, pharmacology, and purification technology, *J of Ethnopharmacology* 306, 116166.

DOST PCAARD, n.d. Bamboo: Industry Strategic Science and Technology Program, retrieved from https://ispweb.pcaarrd.dost.gov.ph/bamboo/ (accessed 21 June 2024)

Galvez, M.C., Cadondon, J., Mandia, P., Macalalad, E., Vallar, E., Shiina, T. (2023). Characterization of Porcine Skin Using a Portable Time-Domain Optical Coherence Tomography System. *Engineering Proceedings* 58(1), 89.

Goto, H., Lagrosas, N., Shiina, T. (2024). OCT Image Analysis of Internal Changes in Leaves due to Ozone Stresses. In *Proceedings of the 12th International Conference on Photonics, Optics and Laser Technology – Volume 1: PHOTOPTICS*; ISBN 978-989-758-686-6, SciTePress, 65-71.

Inskeep, W., Bloom, P. (1985). Extinction coefficients of chlorophll a and B in n,n-dimethylformamide and 80% acetone. *Plant Physiology* 77(2), 483-5.

Ivanova, D. G., Singh, B.R. (2003). Nondestructive FTIR monitoring of leaf senescence and elicitin-induced changes in plant leaves. *Biopolymers (Biospectroscopy)* 72, 79-85.

Li, Y., Scales, N., Blankenship, R.E., Willows, R.D., Chen, M. (2012). Extinction coefficient for red-shifted chlorophylls: Chlorophyll d and chlorophyll f. *BBA Bioenergetics* 1817(8), 1292-1298.

Liu, H., Xiao, C., Qiu, T., et al. (2023). Selenium Regulates Antioxidant, Photosynthesis, and Cell Permeability in Plants under Various Abiotic Stresses: A Review. *Plants,* 12(1), 44.

Meglinski, I.V. Buranachai, C., Terry, L. A. (2010). Plant photonics: application of optical coherence tomography to monitor defects and rots in onion. *Laser Physics Letters* 7, 307-310.

Popescu, D.P. et al. (2011). Optical coherence tomography: fundamental principles, instrumental designs and biomedical applications. *Biophysical Review* 3, 155-169.

Shiina, T., Moritani, Y., Ito, M., Okamura, Y. (2003). Long optical-path scanning mechanism for optical coherence tomography. *Applied Optics* 42(19), 3795-3799.

Wang, S. et al., (2014). Maintenance of Chloroplast Structure and Function by Overexpression of the Rice Monogalactosyldiacylglycerol synthase Gene leads to Enhance Salt Tolerance in Tobacco. *Plant Physiology* 165, 1144-1155.

Yao, X.Y., Liu, X.Y., Xu, Z.G., Jiao, X.L. (2017). Effects of light intensity on leaf microstructure and growth of rape seedlings cultivated under a combination of red and blue LEDs. *Journal of Integrative Agriculture* 16, 97-105.

Zhang, H., Ge, Y., Xie, X., Atefi, A., Wijewardane, Thapa, S. (2022). High throughput analysis of leaf chlorophyll content in sorghum using RGB, hyperspectral, and fluorescence imaging and sensor fusion. *Plant Methods* 18, 60.

Zhao, Y.R., Yu, K.Q., Li, X., He, Y. (2016). Detection of Fungus Infection on Petals of Rapeseed (*Brassica napus L.)* Using NIR Hyperspectral Imaging. *Scientific Reports* 6, 38878.