DOI 10.5162/SMSI2023/P15

Limitations of the fluorescence lifetime measurement from green plants using FD-FLIM to monitor plant health due to prolonged laser exposure

Maximilian Wohlschläger[1], Martin Löder[2], Marti Versen[1], Christian Laforsch[2]
[1] Rosenheim Technical University of Applied Sciences, 83024 Rosenheim, Germany
[2] University Bayreuth, 95447 Bayreuth, Germany
maximilian.wohlschlaeger@th-rosenheim.de

Summary:
Remote monitoring of plant health is essential for environmental research and agriculture. In this contribution, we demonstrate how FD-FLIM is used to measure the effect of prolonged laser exposure on the fluorescence lifetime of chlorophyll from a dandelion leaf, an oak leaf and grass. We describe the limitations of FD-FLIM and propose further experiments to monitor the plant's health status.

Keywords: chlorophyll, plant health monitoring, fluorescence lifetime, FD-FLIM, remote sensing

Introduction

Living plants have sensitive optical signatures, which are advantageous to monitor plants' health, which is affected by a wide range of contaminants. As a result, plant diseases caused by bacteria [1], fungi [2] or other contaminants such as microplastics [3] are getting more and more attention in research.

Currently, standard methods to determine plant functions are near-infrared (NIR) spectroscopy [5], leaf reflectance spectroscopy [6], pulse-amplitude modulated fluorescence [7] and solar-induced fluorescence spectroscopy [8]. Several factors limit the analysis using the four methods directly in the environment. Sunlight, humidity and the distance of the measurement system to the sample affect the measurement. In [4], a promising method was introduced: measuring the fluorescence lifetime at high distances using a high-intensity, ultra-short pulsed laser to gather plant information.

The results in [4] were auspicious, and thus we conducted experiments using frequency-domain fluorescence lifetime imaging microscopy (FD-FLIM) to determine the effects of prolonged laser exposure on the chlorophyll fluorescence lifetime of plants. Therefore, we intentionally damaged the plants by using the highest excitation power and by exposing the plants to laser light for five durations to investigate the effects of prolonged light exposure on the fluorescence lifetime of the plants. In the end, a way is proposed how the fluorescence lifetime could be used to determine the health status of a plant.

Materials and Methods

In frequency-domain fluorimetry, the samples are excited by a sinusoidally or rectangularly excitation laser source. The fluorescence signal caused by the excitation follows the harmonic excitation phase shifted, amplitude damped, and equivalent shifted [9]. A phase-dependent (PD) fluorescence lifetime can be calculated using the measured phase shift by dividing the tangent of the phase shift by the modulation frequency. FD-FLIM adapts the measurement principle of FD fluorimetry but allows an areal measurement of the PD fluorescence lifetime. The fluorescence lifetime is obtained using an FD-FLIM camera, a laser diode having an excitation wavelength of 488 nm and a laser power of 200mW (both from Excelitas PCO GmbH), a PSM1000 microscope from Motic and two optical filters: a band-pass filter in the excitation path and long pass filter in the emission path. Using this setup, 1008x1008 location-dependent PD fluorescence lifetimes of the plant samples are determined in one measurement. The measurement data is evaluated by a Gaussian analysis resulting in expectation values and standard deviation of the areal PD fluorescence lifetimes.

To investigate the effect of prolonged light exposure, the fluorescence lifetime of a dandelion leaf, an oak leaf and grass are investigated. The FD-FLIM measurements are taken after the laser has radiated for t_1 = 0s, t_2 = 2s, t_3 = 4s, t_4 = 6s and t_5 = 8s the measurement spot of the sample surface.

Results

The measured FD-FLIM data is evaluated using the described methods. In Fig. 1, the PD fluorescence lifetimes are plotted against the measurements time t_i.

Fig. 1 shows that the fluorescence lifetime becomes smaller the longer the laser radiates onto the sample surface. Additionally, it can be obtained that laser irradiation results in an exponential decrease in the fluorescence decay time.

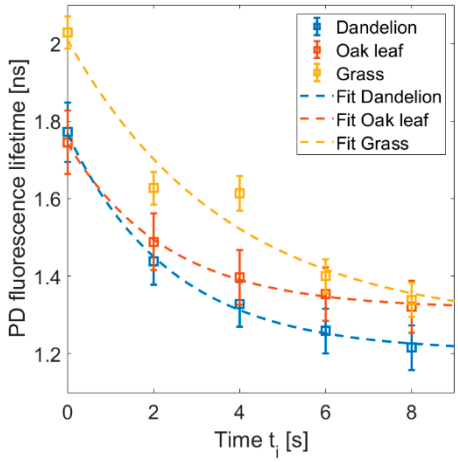

Fig. 1. PD fluorescence lifetime against the time of measurement t_i for the dandelion, oak leaf and grass samples and the corresponding fits.

The exponential model, shown in eq. (1) is fitted to the measurement data, and the parameters for $a\ [ns/s]$, $b\ [ns]$, $c\ [ns]$ and the goodness-of-fit value R^2 are determined and shown in Tab 1.

$$\tau = a \cdot e^{-\frac{t_i}{b}} + c \quad (1)$$

Tab. 1: Obtained values for a, b, c and R^2

Sample	$a\ [ns/s]$	$b\ [ns]$	$c\ [ns]$	R^2
Dandelion	0.56	2.40	1.21	0.99
Oak leaf	0.43	2.29	1.32	0.99
Grass	0.74	3.72	1.27	0.95

The exponential fit shows R^2 values that are larger or equal to 95% which means that the fitted models match the measured fluorescence lifetimes very well. Furthermore, the measured fluorescence lifetimes converge towards a limit value of $c\ [ns] = 1.27 \pm 0.06\ ns$ for long exposure times for the three fitting models. The dandelion and oak leaf show similar values for the parameters $a\ [ns/s]$ and $b\ [ns]$, indicating that they have similar ingredients and chlorophyll content compositions. On the other hand, the values of $a\ [ns/s]$ and $b\ [ns]$ of grass are significantly higher than those of dandelion and the oak leaf, indicating other compounds that influence the fluorescence signal of chlorophyll.

In conclusion, the study showed the exponential decrease of the PD fluorescence lifetime when the leaves are exposed to a prolonged laser light, also known as irreparable photobleaching. The exponential decrease was modelled using the exponential model in eq. (1). A convergence of the PD fluorescence lifetime to $1.27 \pm 0.06\ ns$ is observed for long light exposure times.

Using a high-power laser leads to non-reversible photobleaching of chlorophyll, it is hardly possible to determine the health status of plants. Further experiments must be conducted in which the laser power is reduced so much that no photobleaching occurs. If the inspection of plants is possible without photobleaching effects, the fluorescence lifetime could be a candidate to detect early-stage plant diseases due to the change in chlorophyll and, thus, the fluorescence lifetime.

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