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ABSTRACT

We analyze Terahertz (THz) echoes by reflection on a sunflower leaf in order to evaluate the internal leaf structure (geometry, complex indices and thicknesses). The analysis is based on the thin film multilayer formalism in time and frequency domains. A high agreement is emphasized between experiment and theory, and we evaluate how realistic the multilayer solution can be in regard to our knowledge related to the sunflower leaf. A test campaign is performed in Charles Coulomb laboratory, which is equipped with the THz spectrometer.

Keywords: Terahertz, Reflection, Optical thin layer, Sunflower, Leaf, Absorption, Echoes, Dehydration.

1. INTRODUCTION

Over the past decades, climate change has challenged agronomists to find new techniques and plant varieties genetically suitable to water stress. Indeed, lack of water and drought episode that threaten several regions across the world in the near future has increased number of scientific investigations on new phenotyping tools and techniques. Until now, phenotyping requires statistical analysis so a huge number of data to measure and process. In this context, the availability of massive characterization vectors, like drones, offers new opportunities for the development of optical tools of analysis. For example, multi-spectral and infrared imaging are already operational at the level of the crop. The aim of our study is to develop new optical tools to monitor sunflower plant at the leaf scale.

Time domain spectroscopy has been largely used to probe different materials such as thin homogeneous films or living matter samples considered as a unique layer[1,2]. Some of them have already treated plant leaf case but considering the living tissue as one layer[3]. These studies aimed to estimate water content status of the plant. Most of these methods are based on the theoretical formula of transmission in frequency domain t(f) and of the sample under study.

In this context there is a growing demand to develop non-invasive (optics, acoustics, mechanics…) techniques to analyze leaves microstructure. Among them are optical techniques that have been used for decades to probe elastic and non-elastic properties of the plants. Luminescence properties were extensively explored and mainly provide chemical information (chlorophyll-related) about the samples and photosynthesis, while few information related to the opto-geometrical properties, that is, the thicknesses and indices of the layers which constitute the leaf.

The main idea of this study is to use wavelength in the Terahertz (THz) frequency domain to avoid scattering phenomenon we face using optical or X-ray wavelength. Indeed, roughness of leaf layers are about some micrometers and THz pulses will see them as a flat and homogeneous layers. Hence, it could be possible to model the leaf as interferential filter with absorption.

2. METHOD

2.1 Terahertz Time Domain Spectroscopy (TTDS)

In this study we performed the measurements campaign on a Terapulse 4000 spectrometer (TeraView Ltd., Cambridge, UK) with a femto-second fibre laser light source and a photoconductive antenna emitter/detector. Figure 1 illustrates the principle of measurements. A near-infrared femto-seconds laser pulse (< 90 fs) is first divided with a beam splitter.

One of the beam follows a transmitter line and the other a receiver line. The THz pulse is guided on the sample under study. Receiver is then activated by the same infra-red pulse but with a time offset introduced by the delay line. This operation is repeated many times to rebuild step by step the time spectrum that we will use in our work. For further details, [4] is suggested.

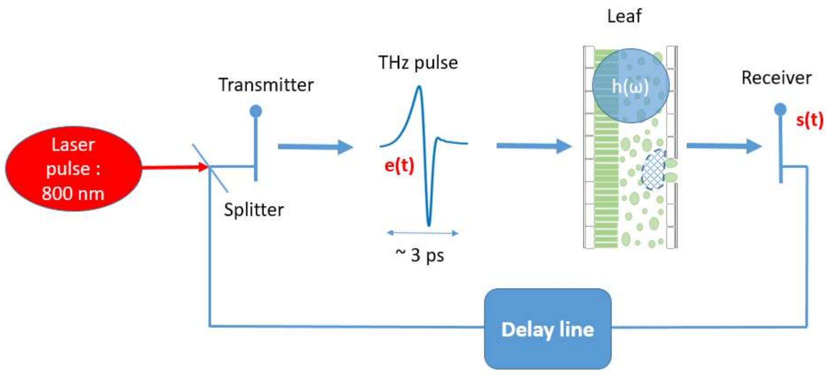

Figure 1 : Simplified representation of a terahertz time domain spectroscopy layout in transmission mode. Transmitter and receiver are both excited by near infra-red femtosecond laser pulse.

Once the reflection is recorded, a numerical fit is performed between optical thin film model and time data in frequency domain. Parameters to evaluate are thicknesses, refraction indices and absorption coefficients. Minimization of such function is performed by a matlab algorithm. The algorithm is guided by a priori knowledge about these parameters that we can find in literature[5]. Figure 2 shows schematically how layers are organized in a dicotyledonous leaf cross-section.

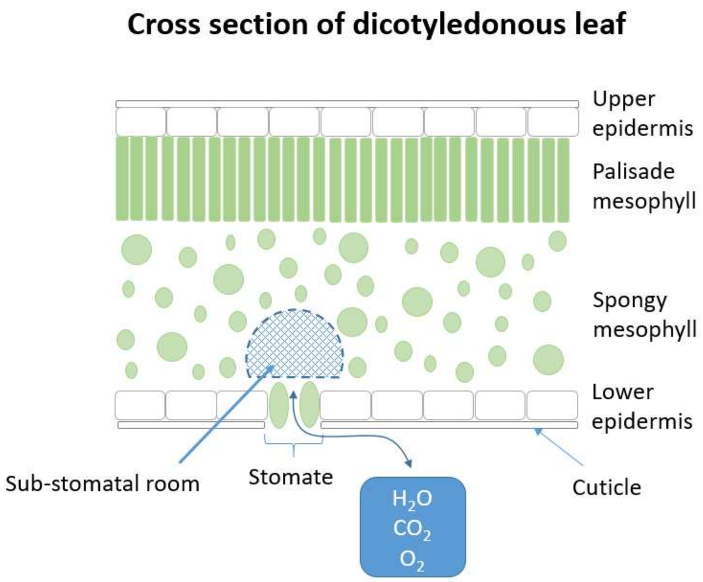

Figure 2 : Classical representation of a slice of a dicotyledonous leaf. From the top to the bottom, we find: upper cuticle; upper epidermis; palisade mesophyll; spongy mesophyll; lower epidermis; lower cuticle. We can add two more layer which correspond to upper and lower trichrome invisible on this scheme.

2.2 Sampling protocol

Different sunflower plants (dicotyledonous plants) with similar size were watered regularly and used for the THz experiments. The plants were grown from seeds in green houses room under log-day conditions in single pots. Plants are enlightened with horticultural lamp for 8 hours a day.

3. DATA

This section presents measurements in reflection we have done on a microscope slide, used as a perfect flat homogeneous layer, and a fresh sunflower leaf. The microscope slide used for the validation of the method is a Thermo Scientific Microscope Cover Slip with a thickness of about 150 µm. Reference in both cases is provided with a gold coated-glass optical mirror. Figure 3 show the plots of these three samples, whose spectra are given in Figure 4.

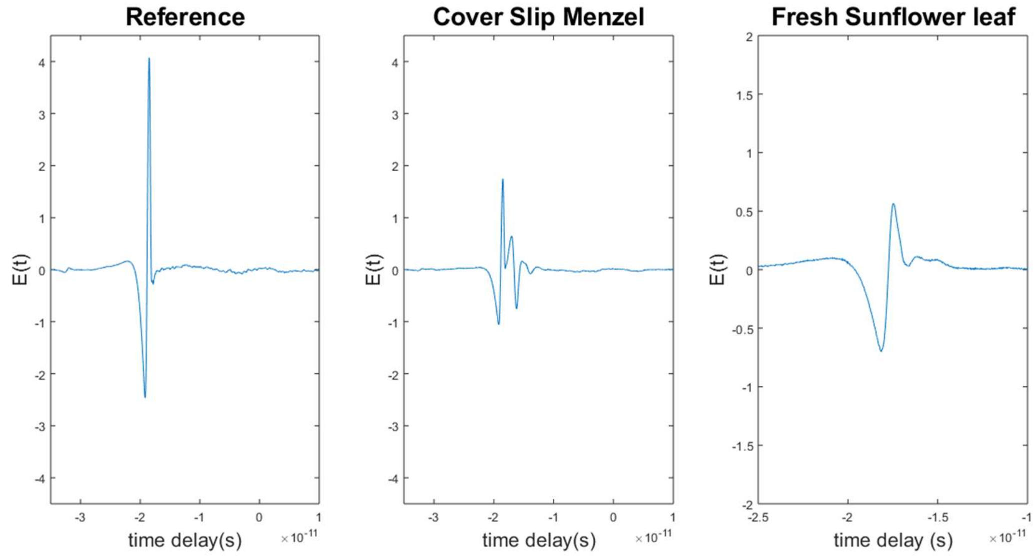

Figure 3 : From left to right, Time spectra of a golden sheet reference, Menzel thermo Cover Slip slide and Sunflower fresh leaf.

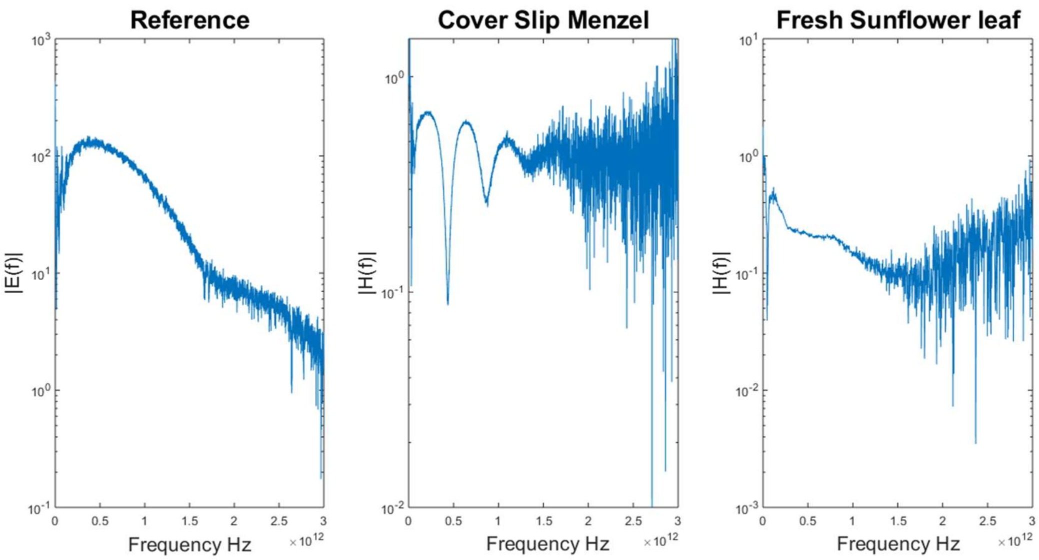

Figure 4 : From left to right, frequency spectrum of a gold coated-glass optical mirror used as reference, Menzel thermo Cover Slip slide, and sunflower fresh leaf.

4. RESULTS

4.1 Results on the microscope slide

This section shows fitting results we obtained with the microscope slide in the reflection mode. Figure 5 shows real and imaginary parts of the frequency spectrum R(f) which are compared with the calculated data issued from the numerical algorithm we used. The right plot then shows how the time-domain spectrum fits the real data in the time space. Values of parameters are indicated in time-domain spectrum plot.

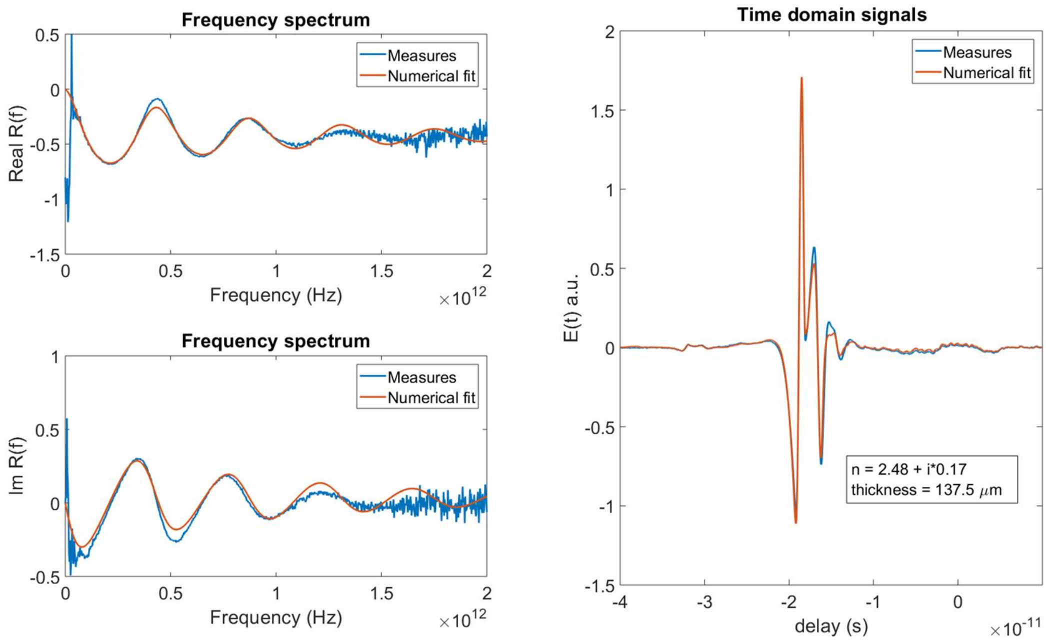

Figure 5: Frequency-domain (left) and time-domain (right) spectra of reflection R(f) data from the microscope slide (blue line) and its fit model (red line). Manufacturer indicates a thickness between 130 and 160 microns.

It is now interesting to compare these parameters estimations with the manufacturing data. Thickness of the Menzel slide was announced to be over an interval of 130 µm and 160 µm. Our thicknesses estimations successfully lie in this range. This allows to extend the method to the study of leaves.

4.2 Results on the sunflower leaf

In the case of sunflower leaf reflection investigation, the THz pulse will be reflected from each layer.. We want to determine how many layers the model needs to fit correctly the time-domain spectra. Figure 6 shows a 8-layer-model simulation compared with the real reflection data. As the previous simulation, parameters estimations are indicated in the time-domain spectrum plot. The right plot corresponds to the frequency-domain in which the fit algorithm has been done from 0.1 to 1.8 THz. Results are successful with 8 layers though we cannot guarantee at this step the unicity of the solution. Note that the number of layers differs from that of Figure 2 (6 layers) because this situation models trichomes layers too.

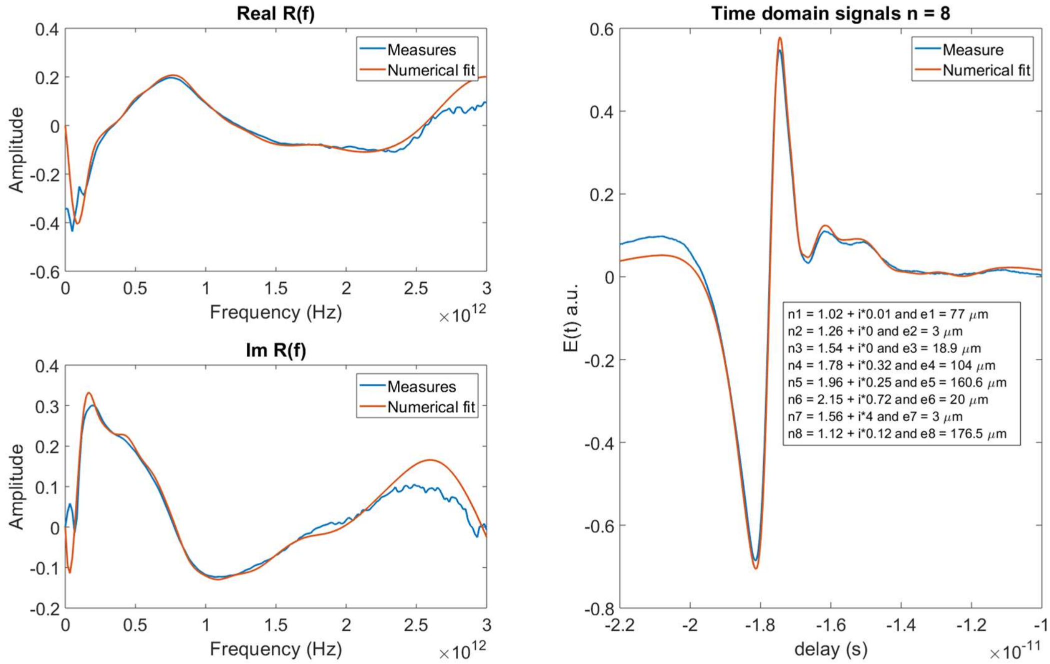

Figure 6: Frequency-domain (left) and time-domain (right) spectra of reflection R(f) data from a fresh sunflower leaf (blue line) and the fit model (red line). Optically estimated parameters from the minimization are indicated in the time-domain spectral plot.

4.3 Discussion

From this simulation fit, a number of remarks must be done. It is clear that, to fit the real time-domain signal in the best way, it is necessary to model the leaf structure with a minimum of layers. In this 8-layers model, both the frequency-domain and time-domain fits are quite good. However, it is important to draw attention to the estimated parameters. Obviously, the fits gets better with the number of layers, but we have to check how the thickness values show agreement with what we know about plant leaf structure. If we look carefully at the estimated thickness values, we somewhat retrieve the characteristic dimensions of the natural layers of a dicotyledonous leaf. Tableau 1 lists all the layer-connection and the estimated parameters associated.

Tableau 1 : Estimated parameters from a 8-layers model. Each layer of the model can be linked to a biologic layer which belongs the natural stack of a dicotyledonous leaf.

Layers	**Correspondence**	**Refraction index (n)**	**Absorption coefficient (k)**
1	Upper trichome	1.02	0.01
2	Upper cuticle	1.26	0
3	Upper epidermis	1.54	0
4	Palisade mesophyll	1.78	0.32
5	Spongy mesophyll	1.96	0.25
6	Lower epidermis	2.15	0.72
7	Lower cuticle	1.56	4
8	Lower trichome	1.12	0.12

We precise here that our model does not take into account dispersion phenomenon. All the estimated values are considered constant in the range of the frequency-domain we work with. Algorythm is improved with few a priori but the values seem to be close to what we can expect. Also, we can notice that the absorption coefficient (k = 4) of the layer number 7 associated with the lower cuticle is out of range. Minimization procedure has to be improved. Furthermore, about the refraction index and absorption coefficient estimation there is no supporting litterature.

5. CONCLUSION

THz time-domain spectroscopy probing leaf has been widely used in transmission to monitor water content. However, in this study, we have exploited reflection data on a leaf sample using THz techniques with the aim of investigating a new method to probe leaf internal structure. This technique uses Optical Thin Layer model to simulate several multilayer stack and compare their reflection coefficient in frequency-domain to the measured data taken on a THz spectrometer. The purpose of this work is to know whether reflection time-domain data are able to provide information on the optical organization of such complex and living sample. After validating the method on microscope slides, a campaign was performed on sunflower fresh leaves. Results of simulations are presented in this paper and show that constraining the solution with some a priori found in literature, allows finding solutions which appear realistic with 8 layers. As can be seen that our technique of optically probing the internal leaf structure leads to promising results, it however can be further improved by numerical algorithm, constrains, model to fit the data. For example, it would be very interesting to include water, air, and dry matter optical properties in THz frequency-domain in the model. Furthermore, new tests measurements have to be performed. These initial results are first step towards the development of a new fast and non-destructive tool to help agronomists and biologists to improve their variety selection method.

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