Research on distribution of leaf water by terahertz spectrum

Jian Zuo (左 剑)*, Zhenwei Zhang (张振伟), Liangliang Zhang (张亮亮), Kaijun Mu (牧凯军), and Cunlin Zhang (张存林)

Key Laboratory of Terahertz Optoelectronics, Ministry of Education,
Beijing Key Lab for Terahertz Spectroscopy and Imaging,
Department of Physics, Capital Normal University, Beijing 100048, China
**Corresponding author: jian.zuoo@gmail.com*

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The influence of varied water distribution in different locations of the mesophyll and mid-vein of the same leaf on the absorption and refraction coefficient is described. And the further comparisons between green leaf and yellow leaf reveal that the complex permittivity of leaf can provide important information about the water content and can characterize the changes of the water distribution of the leaf. So our measurements tend to demonstrate that the dielectric material parameters will be employed to determine the leaf water status in plant leaves.

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The investigation of water status in different leaves is useful for many physicochemical and environ-mental plant physiologists, farmers and botanists. As the plants are not saturated with enough water, they are subject to water stress. It will lead to cell damage and growth inhibition. At present, there is an increasing requirement of fresh water in many countries, so it is beneficial to the irrigation if we can have a good knowledge of the distribution of water content in plant leaves.

There are different methods of determining leaf water status. Normally, these ways can be divided into two types: destructive and nondestructive. In biological lab, the thermogravimetric analyzer is widely used as the measurement, which can provide the high reliability and ease of handling[1]. The content of leaf water is evaluated by the weight difference of fresh and fully dried samples. At the tissue level, these techniques concentrate on measuring the either the energy status of water. They are destructive or preclude continuous observation of water uptake, evaporation and transpiration under non-steady-state conditions. Indirect observation of these parameters is usually performed using porometer that measure the hydraulic resistance or conductance at the leaf surface (usually ignoring boundary layer effects) [2]. The technique of nuclear magnetic resonance (NMR) had been applied for the measurement of leaf water content in lab. However, the large electromagnets and large currents make the construction of compact instrument based on this technique in future almost impossible[3]. So for a long-term research of the leaf water, the nondestructive methods are required. The microwave and infrared spectroscopy are preferable.

The change of the permittivity of the leaf with different water content has been measured using microwave measurements[4]. The dielectric constants of leaves have been given in the range of 100 GHz. However, the permittivity is affected by the salinity of the water in the leaf. Now, the evaluations of water in leaves are being carried out using infrared spectroscopy. The features of reflectance from vegetation foliage upon leaf dehydration are investigated in the near infrared and short-wave infrared with the aid of chemometrics[5].

Recently, the terahertz (THz) frequency region from 0.1 to 10 THz (or 3 333 cm^{-1}) has seen a flurry of research activity in the fields of physics, chemistry and biomolecules. It lies between the mm-wave and far infrared bands. It mainly provides the information about the intermolecular or phonon modes. These modes are usually unique to one particular molecule. So the terahertz technique can be used for the identification of unknown biological or chemical materials. This unique character is also called "THz fingerprint". In particular, THz radiation is non-destructive and non-contact. Terahertz is sensitive to water. In other words, its electromagnetic radiation is strongly absorbed by water molecules. The transmission of leaves usually depends on the total absorption, which depends on the optical path length of water. As a plant is subject to water stress, the path length of water is reduced. However the other part of the leaves is not affected. Accordingly, the absorption measurement can be applied for the evaluations of water content. The THz beam can have a large cross section which covers a large section on the leaf surface. And, the minor angular misalignments between the leaf surface and the incident beam can have a fairly limited effect. So the leaf surface irregularities do not affect the measurements through induction of excessive scattering[6]. The THz frequency is a better window.

Euonymus japonicus is a popular ornamental plant, both in its native area and also in Europe and North America. In particular the numerous cultivars which have been selected are widely grown in all soil types in sun or shade. Here, we obtain the THz spectrum of the leaf "Euonymus japonicus" under two different circumstances where one is the normal leaf and the other one becomes yellow on its surface of the leaf because the chlorophyll is partly destroyed. The refractive index n and the absorption α of the leaf can be available. The values of either n or α can be used for the evaluations of the leaf water content. The THz spectrum of the mid-vein and the mesophyll on the different locations of the lamina are investigated. The water contents changes with the different locations of the leaves. The rules are also discussed.

The leaves belong to Euonymus japonicus and they were biologically kept fresh and live so that the water evaporation with time is reasonably ignored during the measurement. They are with similar sizes and similar heights. One type is green leaf, which is watered as regularly. The other type seems to be lack of chlorophyll and it is yellow color. The thickness of the leaf was given by the way of the micrometer screw. And a gentle turning of the screw assures an undamaged leaf surface. The mass of the leaves were measured by electronic balance. The mass change corresponds to the water loss in the leaf as the time goes. The determination of the leaf thickness represents an indirect measurement of the water content in leaf. But there are errors in determination of the water content in leaves. So the THz spectroscopy can provide a better method.

THz system was described below. Mai-tai laser (Spectra-physics) provided the 100-fs pulses with the central wavelength of 810 nm. The laser pulse was split into pump and probe beams. The pump beam was focused onto the InAs crystal to generate THz pulses. The generated THz pulses transmitted through four off-axis parabolic mirrors (PM) and were focused on a ZnTe crystal with a thickness of 2 mm. The probe beam was focused on the same spot on the ZnTe crystal, where the pump THz pulses focused. The ZnTe crystal was used to detect THz pulses by applying an electro-optic sampling technique. Here, the THz spectrum from 0.2 to 2.6 THz was obtained. The refractive index and the absorption values of the sample are calculated through Fourier transformed[7].

The evaluations of the water content in one leaf is not a good judgment on the leaf water status because there exit some differences between the vein and mesophyll of the leaf. So the THz spectrum of the vein and mesophyll are respectively measured by terahertz time-domain spectrum. The refractive index and the absorption values are extracted from the THz data and the tendencies of the change with the frequency are listed simultaneously. The comparisons are made between the two types of the above instances.

The mid-vein of the leaf is the main transport channel of water material. Thus, the THz spectrums of different spots along the mid-vein are measured by THz-TDS.

As shown in Fig. 1, as the height of the spots along the mid-vein becomes thinner, the slope becomes larger. It means that the absorption is more intense as the spot is near the top of the leaf. For the spot close to the stem, the absorption of THz radiation from the water material becomes small. It indirectly proves that the water content increases from the stem to the top of the leaf along the mid-vein. This phenomenon is opposite of our intuitional presumption. In Fig. 2, the refractive indexes change greatly. Firstly, the peak at about 0.3 THz appears as the thick of the leaf becomes thinner. Secondly, the abnormal dispersion is observed especially for the spots far away from the stem of the leaf. The farer away the spot is from the stem, the larger the slope is.

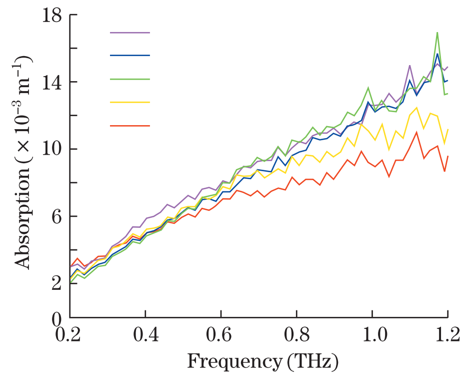

Fig. 1. (Color online) Absorption spectrum of green leaf. The red color corresponds to the thickest spot along the mid-vein of green leaf. The thickness decreases as the color changes from red to purple.

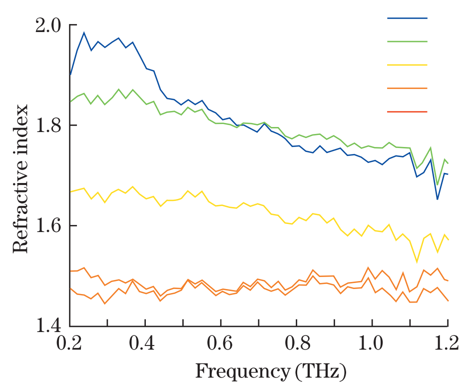

Fig. 2. (Color online) Refractive index of the green leaf. The red color corresponds to the thickest spot along the mid-vein of green leaf. The thickness decreases as the color changes from red to purple.

However, for the mesophyll of the leaf, are there the same relations between the thickness of the leaf and water content? Firstly, the spots along the boundary of the leaf are investigated. In Fig. 3, the absorption changes irregularly. But the values seem to be stable. The slope is similar. We cannot evaluate the water content through the measurement of the boundary values. In the case of the refractive index shown in Fig. 4, the refractive index of the spot near the stem of the leaf behaves differently. The peak marked by red color at 0.22 THz shifts towards 0.6 THz. While the spots are away from the stem spot, the rule of the change of the refractive index with the different frequency is similar. But the water content cannot be properly recognized by the absorption and refractive index of the spots at the boundary of the leaf.

As shown in Fig. 5, thought the yellow leaf losses some water and chlorophyll material, the slopes measured along the mid-vein of the leaf is obviously different. Far away from the stem of the leaf, the absorption values increase. It will pave the way for the evaluations of leaf water status through mathematical functions. These results are consistent with those in green leaf. And at the

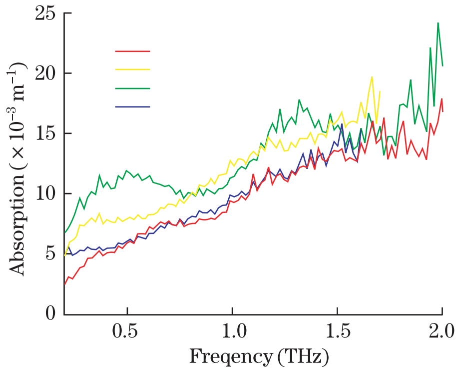

Fig. 3. (Color online) Absorption spectrum of green leaf at the boundary. The red color corresponds to the thickest spot along the boundary of green leaf. The thickness decreases as the color changes from red to blue.

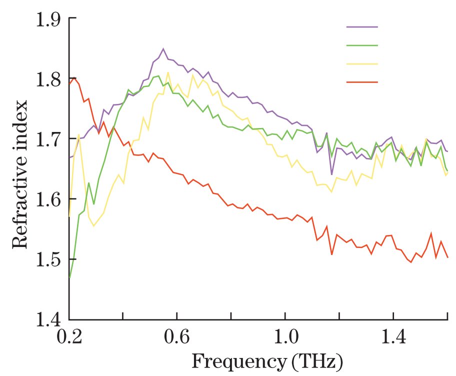

Fig. 4. (Color online) Refractive index of the green leaf at the boundary. The red color corresponds to the thickest spot along the boundary of green leaf. The thickness decreases as the color changes from red to purple.

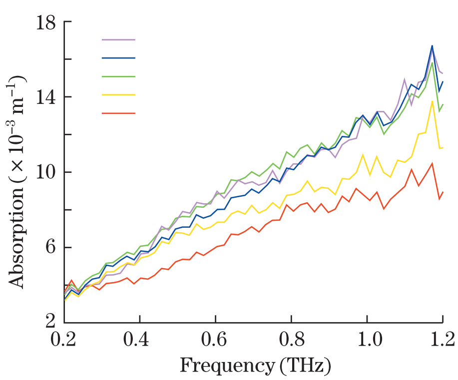

Fig. 5. (Color online) Absorption spectrum of yellow leaf. The red color corresponds to the thickest spot along the boundary of green leaf. The thickness decreases as the color changes from red to purple.

frequency of 1.0 THz, the absorption from the mid-vein of the leaf is usually larger than that form the mesophyll. It is believed that it is necessary to evaluate the water content through the mid-vein of the leaf[8].

In conclusion, we have measured the THz spectrum of the green Euonymus japonicus leaf and yellow Euonymus japonicus leaf. The refractive index and absorption were investigated. It is founded that the water content of leaf makes changes along the mid-vein of the leaf. But in the mesophyll, the discrepancy between the water content and the thickness of the leaf is not evident. It is proved that the water distribution is not homogeneous in one leaf.

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