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Leaf water diffusion dynamics in vivo through a sub-terahertz portable imaging system

F V Di Girolamo^{1,2}, A Toncelli^{1,2}, A Tredicucci^{2,3,4}, M Bitossi^{1,5}, R Paoletti^{1,6}

¹ Istituto Nazionale di Fisica Nucleare, Largo B. Pontecorvo 3, 56127 Pisa, Italy.

² Dipartimento di Fisica, Università di Pisa, Largo B. Pontecorvo 3, 56127 Pisa, Italy.

³ NEST, Istituto Nanoscienze – CNR, Piazza S. Silvestro 12, Pisa, 56127, Italy.

⁴ Fondazione Bruno Kessler (FBK), Via Sommarive 18, 38123 Povo, Trento, Italy.

⁵ European Gravitational Observatory (EGO) via E. Amaldi, 56021 S. Stefano a Macerata, Cascina (PI) , Italy.

⁶ Università di Siena via Roma 56, 53100 Siena, Italy.

E-mail: flavia.digirolamo@df.unipi.it

Abstract. The development of terahertz based technology has given the opportunity for the realization of non destructive techniques capable of gaining meaningful information on delicate systems such as biological samples. Here, the health status of leaves in vivo has been monitored through a portable terahertz imaging system. The data have been extracted and analysed from the images acquired and compared with analogous results reported in the literature on similar systems. The possibility of extracting additional information from the images regarding leaf details has also been explored.

1. Introduction

The interest in terahertz radiation (100GHz-30 THz) is motivated by its potential use in several fields, such as environmental control, medical diagnosis, chemical and biological identification [1, 2]. In the framework of materials identification, the vibrational, rotational and translational responses of molecules and elements in the terahertz range provides informations which cannot be supplied by similar techniques (i.e. optical, nuclear magnetic resonance, X-ray) [1, 2]. Water is markedly well distinguishable since its presence is immediately detectable due to a strong attenuation of the terahertz signal [3, 4].

Water content is the most meaningful datum regarding plants health condition, since it provides an immediate information on illnesses, transpiration, nutrient transport [5, 6, 7, 8], even though in most of the cases it can be measured only by sacrificing the sample [2, 9].

In this regard, since terahertz signal results to be more attenuated if water content is higher and vice versa, it has been consequently exploited for the realization of effective and non-destructive methods to measure water content [10] in a plant [11, 12]. It has been demonstrated that leaf water status and the hydration and dehydration dynamics can be easily locally monitored through terahertz continuous wave or time domain spectroscopy both in removed leaves and in vivo [13, 14, 15, 16]. A decrease in terahertz signal can be associated to hydration, while an increase has been interpreted as dehydration [13, 14, 16]; dehydration dynamics have been fitted with an exponential curve [17].



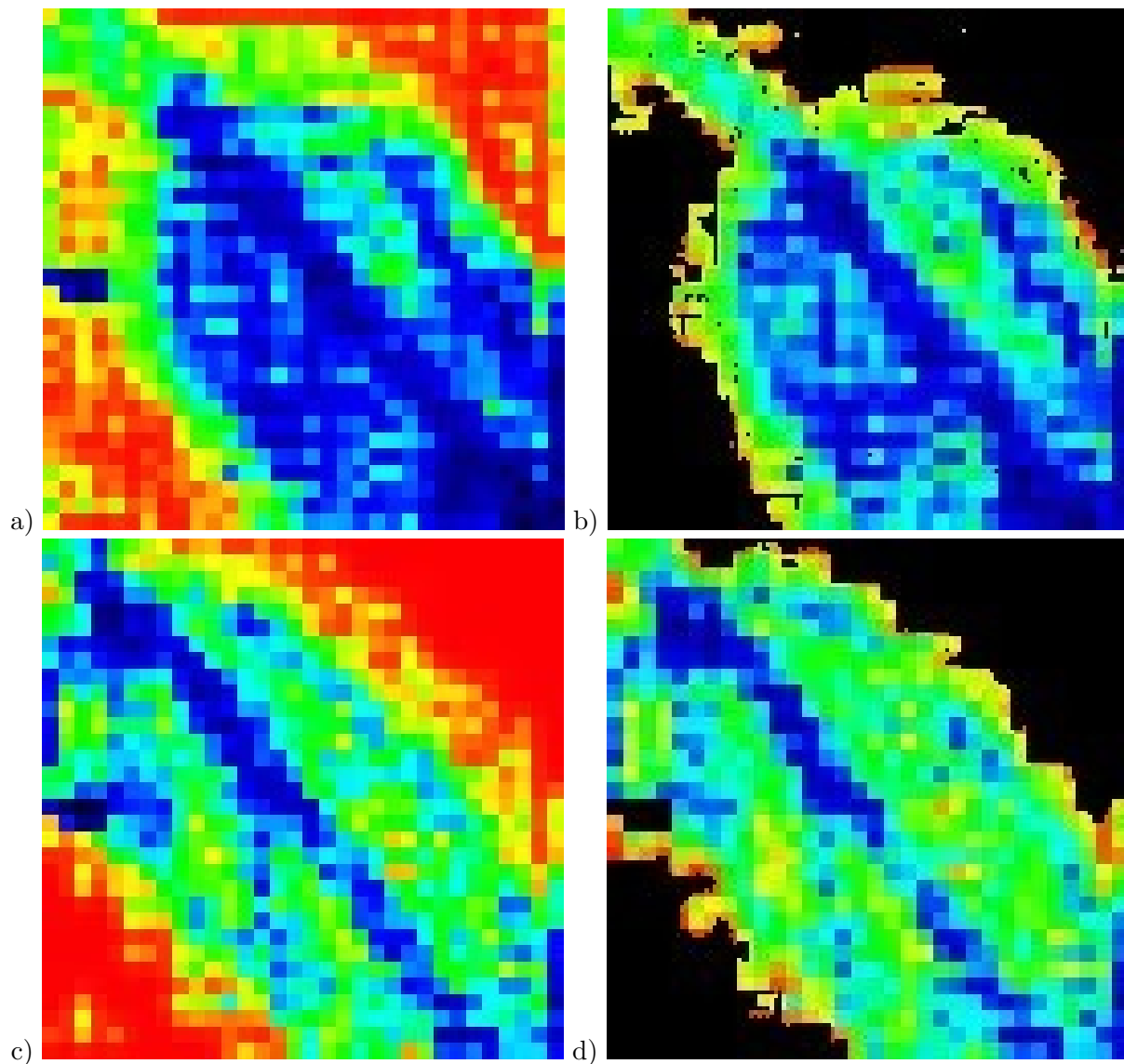


Figure 1. Leaf images acquired with the sub-terahertz imaging system. Removed leaf: (a) original image (b) leaf area. Attached leaf: (c) original image (d) leaf area. The stalk is on the left upper part of the image.

The abundance of information that can be achieved by an experiment immediately grows with the acquisition of terahertz images, rather than single measurements [18, 19, 20]. As a natural evolution of terahertz investigation techniques, an imaging system can be realized by using a continuous wave [19, 20] or a time domain system [16]; high resolution necessary to highlight some details can be achieved by using a quantum cascade laser as source [21].

Here a compact and portable active sub-terahertz imaging system composed by a 100 Ghz source and a camera has been used to study hydration and dehydration dynamics on the whole leaf surface both in removed leaves and in vivo (i.e. leaf still attached to the plant). The leaf image is clearly identifiable from the picture (Fig.1), where different colors also reflect different water concentrations (i.e. blue highest water concentration, red lowest).

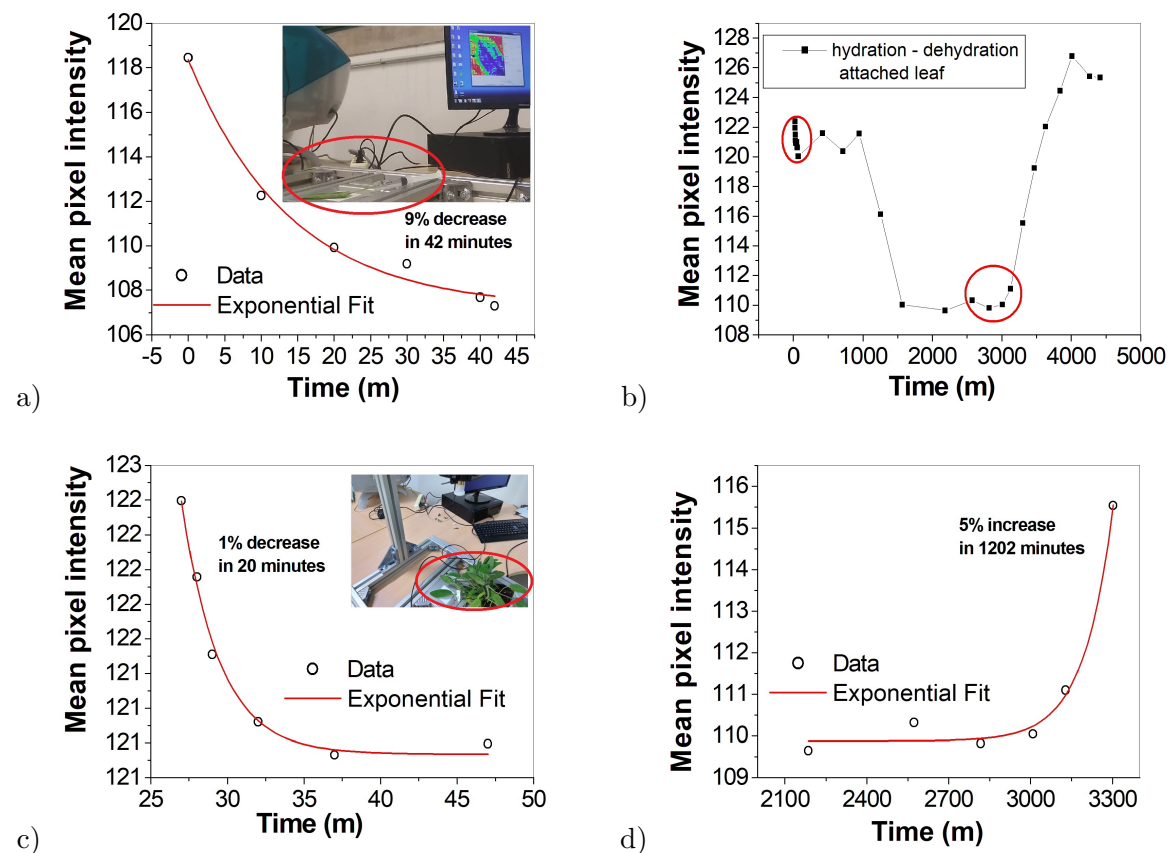


Figure 2. (a) hydration dynamics of a removed leaf; time zero corresponds to water supply. (b) hydration and dehydration dynamics of an attached leaf (time zero corresponds to irrigation; hydration and dehydration exponential regimes are highlighted with red circles): (c) focus on the exponential hydration regime highlighted in Fig.2b; (d) focus on the exponential dehydration regime highlighted in Fig.2b.

2. Materials and methods

The terahertz imaging system was provided by TeraSense company and was composed by an impact avalanche transit time (IMPATT) diode source (generating an output of 80 mW at 100 GHz) and a camera composed by a bidimensional matrix of sensors (1024 pixel). The leaf edges identification and the primary vein selection have been performed by using a software specifically devoted to scientific images analysis (Gwyddion) and a general purpose software (ImageJ).

Long time hydration and dehydration dynamics (Fig.2 and Fig.3a) and leaf primary vein (Fig.3b) have been studied on sage (*Salvia Officinalis*), both on removed and attached leaves.

In the case of removed leaf, the leaf was provided by water by immersing the stalk into a syringe deprived of the needle and filled of water (see inset of Fig.2a). The hydration was supposed to be sufficiently fast to be recorded immediately after the leaf has been put in contact with water, when dehydration could be completely neglected.

In the case of attached leaf, water was provided by irrigating the whole plant. The plant was put close to the system (see inset of Fig.2c); only one leaf was interposed between the source and the detector even if leaves movements (addressable to water diffusion) have occurred, apparently without affecting the overall hydration/dehydration dynamics.

Both for removed and attached leaves water was supplied in the late morning (about 11 a.m.); hy-

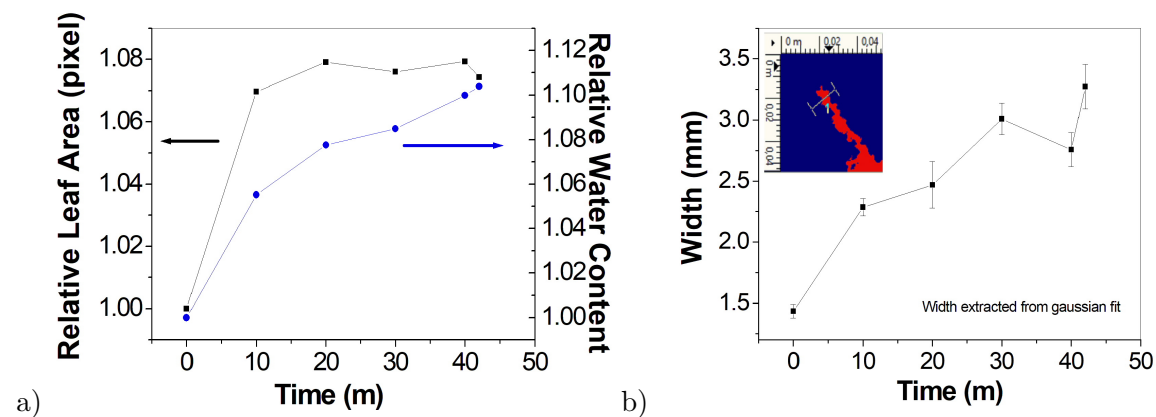


Figure 3. a) Comparison of the relative leaf area and the mean relative water content for the removed leaf. b) Width obtained by fitting the primary vein profile with a gaussian curve for the removed leaf. In the inset, the primary vein area and a profile used for the fit (i.e. from the 10 minutes frame).

dration was performed under room artificial light (a video of the whole experiment was recorded during hydration on the removed leaf), while dehydration in the dark. The leaves were as much as possible of comparable size.

Once the leaf area was selected, the mean pixel intensity was determined, then it was assumed as an indication of the mean terahertz transmission. The mean pixel intensity was then plotted versus the time passed by water supply. Variations of the measured area (i.e. due to leaves movements consequent to water diffusion) give rise to a shift of the mean pixel intensity. The relative leaf area (i.e. leaf area in pixel at time t divided by leaf area at time zero) and the mean relative water content are compared in Fig. 3a. The mean relative water content was determined by an equation derived by the Lambert-Beer law [22]:

$$\text{Mean Relative Water Content} = \ln(I_{\text{blank}}/I(t)) / \ln(I_{\text{blank}}/I(t_0)) \quad (1)$$

where I_{blank} is the mean pixel intensity without leaf, $I(t)$ is the mean pixel intensity with leaf at the current time t , $I(t_0)$ is the mean pixel intensity with leaf at time zero. Lower pixel intensity corresponds to higher attenuation of the terahertz signal and higher water content, and vice versa.

In the central section of the leaf a continuous line was evident, which mostly coincides with the primary vein. Even if it probably includes also some small secondary veins, an effort has been spent on the selection of its area and on the estimation of its width, in order to provide a tool for the identification of leaf details (i.e. such as in case of plant diseases). The profile perpendicular to the primary vein direction was averaged on the central section of the leaf (in order to avoid the stalk and the tip). The resulting profile was fitted by a gaussian function and the width was plotted (Fig.3b) versus the time passed by water supply.

3. Discussion

In the case of the removed leaf, the mean pixel intensity decreases soon after water supply, suggesting a water content increase (Fig.2a) This agrees well with the literature, where a similar behavior was ascribed to a hydration dynamics. Mean pixel intensity decrease has been fitted with an exponential equation (fitting parameters reported in Tab. 1):

$$y = y_0 + A \exp(-x/t) \quad (2)$$

In the case of the attached leaf (Fig.2b), the mean pixel intensity also decreases soon after plant watering, then it maintains approximately constant (except for a shift which can be ascribed to leaves movements) and then it increases again (i.e. suggesting dehydration beginning to occur). An exponential regime can be identified soon after plant watering (Fig.2c), which can be addressed to hydration dynamics as well. It has been also fitted with equation (2) (fitting parameters also reported in Tab. 1). It seems to be slower than the case of the removed leaf.

Another exponential regime can be individuated at 42 minutes after irrigation (Fig.2d), and can be consequently interpreted as dehydration dynamics. It is highly slower than the hydration phenomenon, occurring on a time scale of hours instead than minutes. It has been fitted with an exponential equation (fitting parameters reported in Tab. 1):

$$y = y_0 + A \exp(x/t) \quad (3)$$

hydration flux in the leaf is not uniform throughout the overall area: for instance, it can be different in the distal respect to the intermediate and the basal leaf regions [16]. Moreover, monitoring the size variation of eventual defects present on the leaf could be useful for the detection of diseases (somehow in analogy with medical diagnosis) [9].

Here, since the plant was basically healthy, the attention has been focused on the region around the primary vein, and, in particular, on the variation of its width during hydration. The width, estimated with the procedure reported in the previous paragraph, seems to increase during hydration, as well as the leaf area and the water content (Fig.3). It is worth to mention that the leaf area does not necessarily correspond to the real leaf area (whose size variations are expected to be minimal [22]) but to the area of the leaf where water concentration is sufficiently higher than the background. Analogously, the increase in primary vein width is expected to simply reflect an increase of the water content in the primary but also secondary veins, rather than a real increase in its diameter. Further investigations in this direction will provide more detailed information.

Table 1. Fitting parameters.

	y_0	A	t (m)	$red.\chi^2$
Removed leaf	107.2±0.6	11.2±0.7	14±2	0.99
In vivo (hydration)	120.95±0.04	226880±303992	2.3±0.2	0.99
In vivo (dehydration)	109.9±0.2	(1.6±9.9) * 10 ⁻¹³	106±21	0.99

4. Conclusion

Long time hydration and dehydration dynamics have been monitored through a terahertz portable imaging system both in a removed leaf and in vivo. After leaf/plant watering a

decrease in terahertz signal has been detected, in agreement with the literature [13, 14, 16]. The removed leaf exhibits an exponential decay. For the attached leaf two exponential regimes have been detected: an exponential decay few minutes after plant watering (identifiable as hydration dynamics) and an exponential growth 42 hours after plant watering (identifiable as dehydration dynamics). Some additional information have been extracted from the data regarding the leaf area, the water content and the primary vein width variations. Those parameters all slightly increase with time soon after water supply, as a direct consequence of the increased hydration of the plant.

Acknowledgments

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