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Monitoring of vegetation drying by Brillouin and Raman spectroscopies

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ABSTRACT

Raman and Brillouin spectroscopic provide with a powerful way to non-invasively assess both chemical and physical (viscoelastic) properties. In this report, Brillouin microspectroscopy was used for real time analysis of elastic properties of *Populus* and *Geranium* leaves, while Raman spectroscopy and imaging were employed for assessment of their chemical variation during drying. When used together, those techniques can improve our understanding of mechano-chemical changes of plants in response to environmental stress and pathogens at microscopic (cellular) level. Our results have demonstrated for the first time the ability of multimodal assessment of elasticity modulus, hydraulic conductance and interatomic vibrational modes in plants as emerging new markers for real time quantitative assessment of agricultural crops.

Keywords: Brillouin, Raman, elastic modulus, hydraulic conductance, Populus, Geranium, leaves

1. INTRODUCTION

The world population is expected to reach 10 billion people by 2050, and farmers all around the world will need access to tools and technologies to help feed a growing population, while at the same time protecting our environment, making it the greatest challenge faced by global society. Pathogens are the single largest threat to a crop, accounting for up 40% losses world-wide making it a $500 bln per year problem [1]. Optical imaging and sensing technologies have demonstrated a high potential for remote and near-range disease detection and crop health monitoring; however, it has also been widely recognized that a multimodal approach with a closer link between molecular diagnostics and imaging methods would contribute both to a better understanding biochemical and biophysical processes during disease development and provide a much better interpretation of the imaging data [2]. Clearly, such approach would require a synergetic effort in several areas, such as plant pathology, optical engineering and informatics, and this proposal uniquely combines multidisciplinary expertise of several international groups to tackle the above grand-challenge. One particular challenge is related to developing new tools and, more specifically, optical imaging methods, which are capable of providing more information on the state of plants' health. Traditionally, physiological performance of agricultural crops over a large area is done by thermal aerial mapping [3]. However, thermal imaging is difficult to accomplish with a high spatial resolution, and results often depend on atmospheric conditions. Thermal imaging does not necessarily yield critical quantitative mechano-chemical information related to the plants health. In contrast, Brillouin and Raman spectroscopies offer potential for quantitative remote, noninvasive and real-time monitoring of plants vegetation by measuring plants elasticity and chemical composition in response to environmental stress and pathogens.

The purpose of this experimental study was to explore the feasibility of using mechano-chemical properties of plant leaves and their evolution while drying as a potential marker, which can be subsequently assessed using Brillouin and Raman spectroscopy.

Brillouin light-scattering microscopy is an emerging spectroscopic technique for investigating biomaterials *in situ* and *in vivo* [4-5]. It is based on the interaction between laser light and inherent thermal vibrations (acoustic phonons) in the biomaterial under study. This interaction produces a small part of the inelastically scattered light. When a sample is

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illuminated with a single-frequency laser source, this inelastically scattered Brillouin light photons are readily detected being spectrally shifted by the frequency of acoustic phonons. This frequency shift is called Brillouin frequency shift (BFS), and it is related to the longitudinal elastic storage modulus (M') of the investigated biomaterial [6-7].

Applications of Raman scattering, which is considered to be a chemically-specific spectroscopy technique, for characterization of plants have been reported in a number of publications (see, for example, [8-9]). Typically near-IR lasers with the center wavelength of 1064-nm were used for Raman excitation to characterize and image various plant samples [10-11]. However, to the best of our knowledge, no reports exist of dynamic changes of Raman spectra (and, subsequently, chemical and/or structural composition) of plants or leaves upon drying. This provided a motivation for this work, which aimed at exploring the potential of multimodal imaging by combining Raman and Brillouin microscopies (see, for example, [12]) for assessing and imaging tree leaves during the process of drying.

2. METHODS

2.1 Materials

After several trials of using various plants' leaves, Populus leaves were chosen for observing changes of elastic properties of drying leaves. The choice was made based on leaves' optimal thickness and its transparency. The same factors were taken into consideration when choosing leaves of Geranium.

We plucked Populus leaves from living plants growing outside. Geranium leaves were plucked from domestic living plant and some of them were probed right from the pot. All the leaves were thoroughly washed with water from dust and other possible contaminants. The plane of the explored leaves was normal to the incident laser beam as shown in Figure 1.

Figure 1. Laser illumination of living Geranium leaf under microscope objective in Brillouin spectroscopy experiment

2.2 Brillouin spectroscopy

Brillouin scattering setup consisted of a light source (Laser Quantum's Torus 532 nm single longitudinal mode laser), a sample on an orientation device, confocal microscope with high-contrast analyzer based on 6-pass scanning tandem Fabry-Perot interferometer equipped with single photon counting photodetector (TFP-1, JRC Scientific instruments).

Monochromatic laser light was focused on a plant leaf. Interacting with acoustic phonons, a part of the incident light causes Brillouin Stokes and Anti-Stokes scattering [6]. Brillouin light scattering spectra were measured from the drying Geranium leave and from living Geranium leaf during 24 hours, and from drying Populus leaf during 12 hours.

2.3 Raman spectroscopy

Two poplar leaves for two trails were picked up with interval of 2 weeks, taped between 2 microscope slides weighed on analytical balance and observed by Raman microscopy in 0, 3, 6, 9, 12, 24, 27 and 30 hours since they were picked up. Raman mapping is done with Horiba LabRAM spectrometer with 532 nm excitation, using microscope with the 10x objective, 2 sec integration time and 2.5% laser power. The level of incident laser power was empirically determined to minimize any possible damage to the sample. The average of 3 maps, 25 point each were averaged for both leaves for every reported time.

During the experiment, the increase of Raman frequency shift and vibration peak within time was detected in drying Populus leaves, when constant values of living Geranium leaves were received.

3. RESULTS AND DISCUSSION

3.1 Brillouin scattering

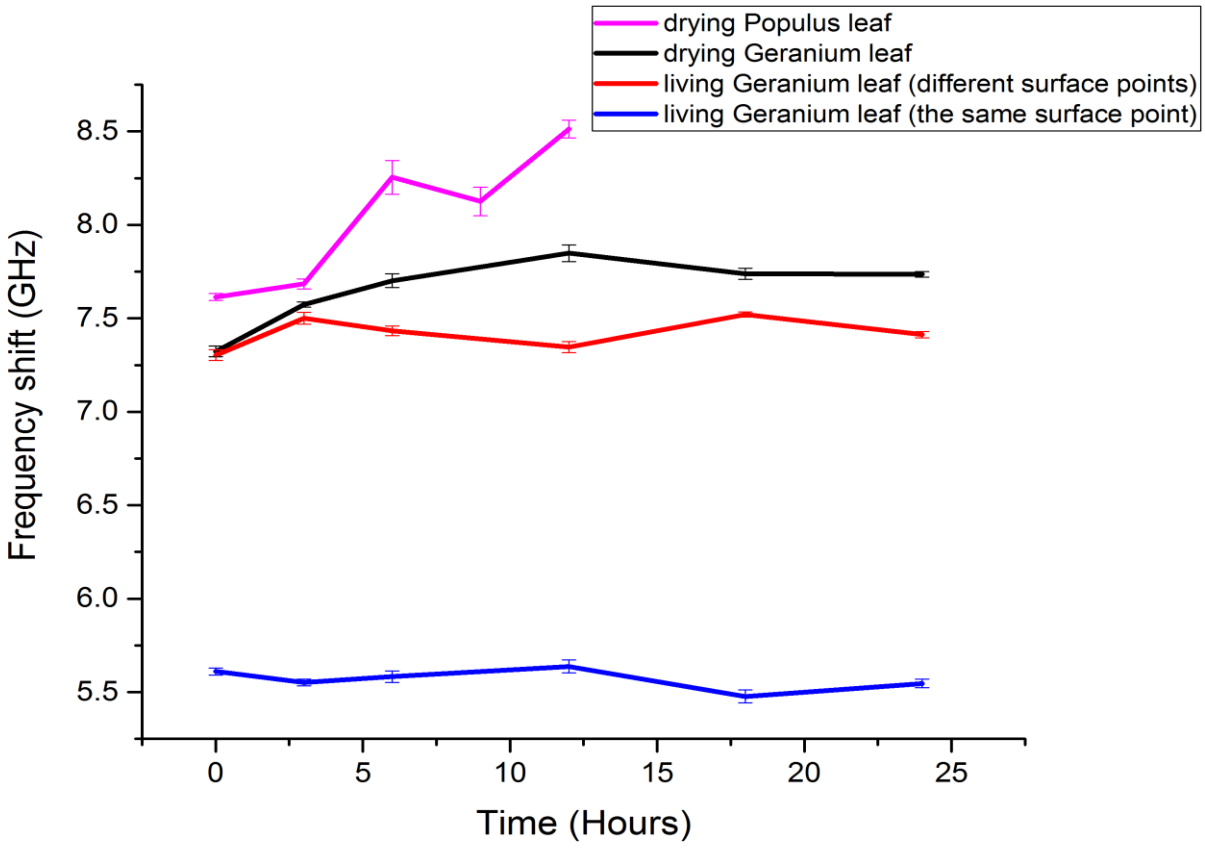

Figure 2. Measured time-dependent Brillouin frequency shift of drying leaves.

As it can be seen from Figure 2, although frequency shift value from *Populus* leaf's scattering fluctuates over time, there is an increasing general trend. Moreover, this graph shows that frequency shift from *Geranium* leaf scattering was growing constantly during first 12 hours, and then started to decrease slightly.

Using

$$\Delta\omega = \frac{2n}{\lambda}\vartheta \cos\frac{\theta}{2}, \quad (1)$$

where Δω is a frequency shift, λ is the wavelength of the incident light, n is the refractive index of the sample, θ is the angle between incident and scattered light, and ϑ is the acoustic velocity, and

$$\boldsymbol{\vartheta} = \sqrt{\frac{\boldsymbol{M'}}{\boldsymbol{\rho}}}, \tag{2}$$

where *M'* is the longitudinal elastic storage modulus, and ρ is density of the sample, the longitudinal elastic storage modulus has been calculated [4]:

$$M' = \boldsymbol{\vartheta}^2\boldsymbol{\rho} = \frac{\Delta\boldsymbol{\omega}^2\boldsymbol{\lambda}^2}{4\boldsymbol{n}^2\boldsymbol{cos}^2\frac{\boldsymbol{\theta}}{\boldsymbol{2}}}\,\rho. \tag{3}$$

The value of the leaves' refractive index is approximately 1.6 [13] and the densities of leaves are about 330 kg/m^3 [14]. The results can be seen in Table 1.

Table 1. Longitudinal elastic storage modulus values obtained during the experiment

	M' of a fresh sample, MPa	*M'* of a dried sample, MPa
Populus	539±170	676±215
Geranium	489±155	545±174

Elastic properties of leaves depend on the type of plant. Moreover, there are several indexes characterizing elastic properties of leaves. In the previous studies, it has been investigated that Populus leaves have connection between the modulus of elasticity and hydraulic conductance. To be more precise, with the decrease of hydraulic distance the elastic modulus increases. It means that when a leaf loses its water, i.e. dries, it has high density and high content of rigid components [14]. We observed the same phenomenon that has been confirmed by the obtained results: the longitudinal elastic storage modulus has increased from 539 MPa to 676 MPa that verified the growth of elastic properties.

Figure 2 also illustrates the frequency shift dependence of living Geranium leaves with time. The difference between these two leaves is just the investigated area of lamina (leaf surface). In the first case, the measurements were taken randomly from different points of lamina, i.e. from the bottom and the top of a leaf, that's why the value of frequency shift was fluctuating. As it was shown in the previous calculations (3), the longitudinal elastic storage modulus is directly proportional to the square of the frequency shift. Therefore, it can be concluded that the value of the longitudinal elastic storage modulus fluctuates from base to tip of a leaf. In the second case, the measurements were taken from relatively small limited area of leaf top such that it can be assumed as one surface point. The graph of living geranium leaf's (the same surface point) scattering frequency shift dependence on time in Figure 2 displays a constant line. Consequently, the longitudinal elastic storage modulus stayed stable within this small defined area of leaf surface. The obtained results agree with the previous studies. All the values indicating the mechanical properties of leaves reduced from base to tip, except for failure strain which was stable [15].

3.2 Raman scattering

Table 2. Characteristic Raman peak area changes upon leaf drying

Time, hours	0	3	6	9	12	24	27	30
Mass, %	**100**	**91.82**	**82.46**	**73.24**	**64.67**	**50.67**	**38.83**	**36.49**
Peak, cm^{-1}								
485	1	1.13	1.32	1.33	1.52	4.08	3.80	4.95
1007	1	1.06	1.11	1.08	1.48	2.26	2.28	2.73
1158	1	1.07	1.14	1.12	1.51	0.87	0.94	0.72
1190	1	1.07	1.15	1.13	1.53	0.65	0.72	0.62
1525	1	1.04	1.08	1.07	1.45	1.51	1.57	1.71
2433.81	1	1.16	1.40	1.55	1.65	1.77	1.42	1.46

As demonstrated in Table 2, there is a general trend of increasing background (BG) adjusted (when fluorescent BG is subtracted from Raman signal) Raman peak area upon leaf drying. In particular, the biggest, about 5-fold increase in Raman peak area is observed for 485 cm^{-1} peak that can be attributed to ring vibrations of starches containing amylose and aminopectin, [16] when the leaves are dried for 30 hours and lost about 63% of their initial weight.

4. CONCLUSION

We provided the results of Brillouin spectroscopy where frequency shift value grows within the time. Populus and Geranium leaves were harvested and plucked for the study. Brillouin scattering measurements were taken every three hours until the plucked leaves were completely dried out accompanied by the increase of stiffness, as evidenced by the rise of the longitudinal elastic storage modulus (M') by 138 MPa and 57 MPa, respectively. Brillouin spectra taken from the same point of living Geranium leaf surface has not revealed any changes in elastic properties.

In Raman scattering, there was a similar ranking of increasing peak area upon drying among: 485, 1007, 2334 cm^{-1} vibration as one described on the previous page. Raman spectroscopy assessed at 3, 6, 9, 12, 24, 27 and 30 hours after plucking the Populus leaf, has demonstrated 5-fold increase in the area of 485 cm^{-1} peak, attributed to the increase of amylose and aminopectine. 1007 cm^{-1} peak attributed to C-H bending increased 2.7-fold. Overall those significant changes in peak ratios indicate that in a future it may be possible to evaluate the drying of plants (trees in the forest or trees, bushes in the city park) using peak ratio analysis of Raman spectra.

Finally, leaves' refractive indexes and density values might be measured while drying using spectroscopy technique in order to get more precise results in the future.

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