Detecting Genetic Variation in Plants by Mapping Cell Water Dynamics With Terahertz Laser Feedback Interferometry

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***Abstract*—This study investigated the use of terahertz (THz) imaging as a rapid, high-fidelity technique for discriminating between genetic variants of the *Allium* genus based on cellular water dynamics. It has been demonstrated earlier that plant genetic variations can be related to the biochemical and biomechanical alterations of the cell and that in turn affect the water dynamics within the cell. In this article, we show that the water dynamics, when considered in the form of the temporal evolution of the trajectory of the plant's response to THz radiation probe, and measured by a coherent THz transceiver, provides unique signature of the genetic makeup of the plant. Therefore, by exploring these trajectories, we discriminate between closely related variants of the same genus. The technique used for THz probing was the laser feedback interferometry with THz quantum cascade lasers, which enabled fast acquisition of high-resolution THz amplitude and phase images, which were processed into evaporation profiles describing the time-dependent dehydration of the samples. The trajectory of this profile in amplitude–phase reflectivity domain discriminates between different members of the *Allium* genus. This enables real-time genetic discrimination in agricultural and genome conservation applications.**

Manuscript received 16 November 2023; revised 15 April 2024; accepted 6 June 2024. Date of publication 11 June 2024; date of current version 5 September 2024. This work was supported in part by the Australian Research Council (ARC) under Grant DP200101948 and Grant DP210103342, in part by the Defence Research and Development Organization (DRDO) under Grant DFTM/03/3203/M/01/JATC, in part by the Department of Atomic Energy - Board of Research in Nuclear Sciences (DAE-BRNS) under Grant 37(3)/14/01/2016-BRNS/37015, in part by the Engineering and Physical Sciences Research Council (EPSRC U.K.) under Grant EP/T034246/1 and Grant EP/W028921/1, and in part by the European Cooperation in Science and Technology (COST) Action under Grant CA21159. *(Urbi Kundu and Jari Torniainen contributed equally to this work). (Corresponding author: Aleksandar D. Rakić)*

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Color versions of one or more figures in this article are available at https://doi.org/10.1109/TTHZ.2024.3412826.

Digital Object Identifier 10.1109/TTHZ.2024.3412826

***Index Terms*—Agri-photonics, laser feedback interferometry (LFI), terahertz (THz) imaging, THz quantum cascade laser (QCL).**

I. Introduction

THE United Nations (UN) Intergovernmental Panel on Climate Change finalized its sixth assessment cycle in March 2023, which highlighted the consequences of the global temperature increase of 1.1°C. The rise in temperature is expected to result in unpredictable weather patterns, sea level rise, water scarcity, limited agricultural advancements, and the extinction of local species [1]. This, compounded with the population growth estimates by the UN Department of Economic and Social Affairs (i.e., approximately 9.7 billion by 2050) [2], paints a grim picture for agricultural food security and the conservation of the available gene pool in the coming years [3].

Food security can be reinforced by selecting specific cultivars that are more resilient to the environment than a more diverse population. The potential adverse effect of the selected cultivars, however, is the reduced capacity to adapt to new conditions posed by factors, such as climate change or sudden onset of disease [4]. Furthermore, concentrating on a particular cultivar can cause a significant loss in the gene-pool variability, which might have been crucial for meeting future food challenges [5]. It has thus become even more critical to conserve plant genetic diversity. However, the need for sustainable food security has prompted the development of genetically modified (GM) crops as a form of crop enhancement. Globally, 32 crops with 525 distinct enhancements, including increased nutritional value, tolerance to biotic and abiotic stresses, and resistance to pesticides, have received approval. These advancements have made a significant impact on the environment, with reduced CO_2 emissions, a 22% increase in crop yield, and a 68% increase in crop value [6]. Alongside the efforts to develop GM crops, scientists are also actively engaged in studying genetic variability and conserving plant genetic diversity [7]. The ability to rapidly and reliably determine the genetic variability of different plant species is vitally important because not only will that allow people and nations to make informed decisions on what cultivars are suitable for their needs but also will assist conservationists in maintaining a diverse gene pool.

Efficient and accurate study of genetic variation and discrimination necessitates rapid and reliable methods. Polymerase chain reaction and enzyme-linked immunosorbent assay [8] have traditionally been the most common techniques. Other approaches, such as high-throughput genome sequencing [9] and chemometric analysis, as well as various microscopic and spectroscopic techniques (e.g., fluorescence, confocal, Raman, and Fourier transform infrared spectroscopy), have been employed to explore genetic variations, biochemical changes, and cellular dynamics [10]. However, these methods often require extensive sample preparation, have limited sample lifetimes, lengthy processing times (e.g., several days), and rely on external probes [11], [12], [13], [14], [15], [16], [17]. Furthermore, the anisotropic nature of biological cells and the time sensitivity of cellular dehydration during sample preparation pose additional challenges. Despite their high fidelity, these methods are hindered by their drawbacks [7], [18], [19], [20], [21], [22]. A method for mitigating these drawbacks could potentially be found in the electromagnetic region between the microwave and infrared, known as terahertz (THz) radiation. THz radiation encompasses the frequency range of 0.1 ($\sim$3000 μm) to 10 THz ($\sim$30 μm) and has been found to have several interesting properties when it comes to studying biological molecules [23], [24].

THz radiation has low photon energies, which means that it is nonionizing and does not induce genetic mutations [25], [26] unless the sample is subjected to high intensity pulses [27], [28], [29]. It exhibits unique spectral fingerprinting due to molecular resonances and is highly sensitive to molecular isomers [30]. The most important biofriendly feature of this range, however, is the fact that THz is highly absorbed by liquid water, which is the most ubiquitous molecule in all biological systems. Thus, the presence of liquid water can act as a natural "biomarker" in THz range, which has already been utilized in several studies of hydration dynamics in the agri-food sector [31], [32], [33], [34], [35].

As a result, THz spectroscopy and imaging techniques are quickly becoming a standard probing tool for studying biological systems [36], [37].

Our research focused on utilizing laser feedback interferometry (LFI) with a THz-based quantum cascade laser (THz QCL) [23], [38], [39]. The system was used to analyze tissue structure and dynamics over time, specifically for identifying genetic variation in closely related species by correlating species specific response of real-time changes in the water content. This imaging technique—compared with more conventional THz time domain spectroscopy—is more compact (due to the sensor being a transceiver with identical transmit and receive paths), is of higher power, and has fast pixel acquisition times. It also enables the concurrent extraction of amplitude–phase data from the sample and allows for rapid high-resolution image generation through raster scanning that has previously been applied to various fields [40], [41], [42], [43], [44], [45], [46].

Since earlier studies have shown that genetic variation can affect the cellular biochemistry and biophysics, thereby influencing the biomechanical aspects of the cell, the current study presents an interesting avenue to investigate the differences brought upon by the genetic variation between different cultivars. To observe the differences in cell water dynamics, we examined onion, leek, and shallot cells (see Fig. 1). This provided us with a range of cell sizes from different cultivars, including cells of similar size from different cultivars. This allowed us to consider a number of different factors related to the cell structure and correlate their corresponding contributions in our present study. With THz spectroscopy, our present hypothesis of correlating genetic variation with the biomechanical response of the cells could be extended to cells of closely related organisms to see if the difference is significant enough to be captured and resolved in a manner that could be further extended to perform discriminatory studies between genetic variants [31], [32].

A critical aspect of ensuring data consistency in THz spectroscopy experiments involves meticulously controlling the sample characteristics. This study meticulously optimizes the age and planar spread of the plant cell samples to minimize experimental variability. Furthermore, it acknowledges and addresses the potential influence of cell orientation within the sample, a factor known to introduce heterogeneity in the collected data. Through meticulous control of these parameters, the study establishes a robust foundation for employing THz spectroscopy as a reliable tool for detecting subtle genetic variations between closely related plant species.

II. Water Dynamics in Plant Cells

For any living organism, its cell is its basic building block. It is the structural, functional, and biological unit of life. It consists of various cellular organelles and biomolecules contained in its cytoplasm, which carries liquid water as its major constituent. It helps in maintaining the shape of the cell by contributing to the formation of the plasma membrane as well as maintaining osmotic balance (see Fig. 2). Further, the liquid water assists in maintaining the cellular pH and acts as a buffer for various biochemical processes [47]. Given the complex nature of the cell and the extensive role that water plays in the same, it is expected that a change in water dynamics within the cell would result in biochemical as well as biomechanical alterations within the cell [48].

In this article, we use the combination of high resolution and high frame rate of LFI–THz–QCL imaging that enabled the detection of minute changes in water dynamics of the measured samples. The analysis of this dynamics allows for the discrimination between closely related variants of the same genus.

III. Materials and Methods

A. Sample Preparation and Experimental Setup

All samples used in the study were extracted from locally sourced specimens of the *Allium* genus, which included four different types of plants: 14 leeks (*Allium ampeloprasum*), 5 shallots (*Allium oschaninii*), 23 pigmented onions (*Allium cepa*), and 23 nonpigmented onions (*Allium cepa*). Each sample was measured in triplicates (i.e., $n = 3$ per type). For each sample, a section measuring 1 cm $\times$ 1 cm of the bulb was excised using a scalpel, and then the underlying epidermis was gently peeled off using a pair of forceps (see Fig. 3). THz imaging was

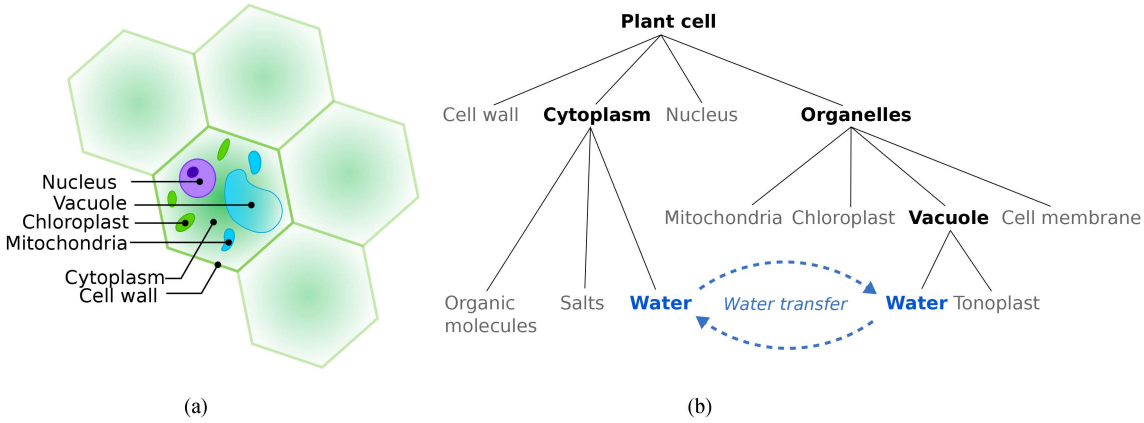

Fig. 1. (a) Representative plant cell. (b) Schematic representation of contents within a typical cell.

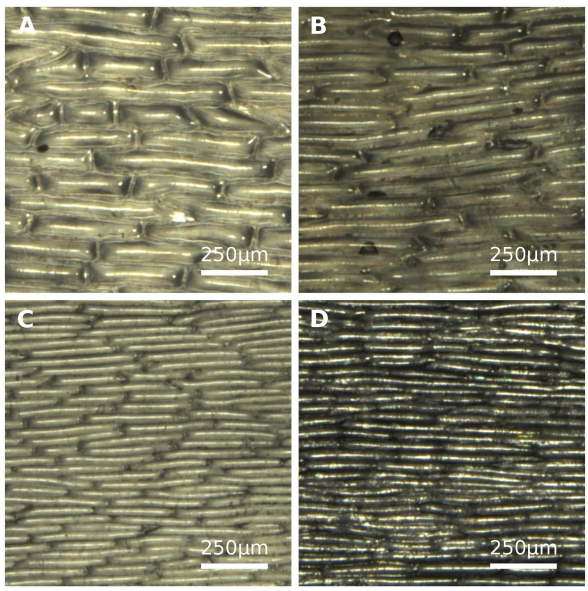

Fig. 2. Microscopic images depicting variation in cell dimensions for epidermal tissues in (A) pigmented (red) onion, (B) nonpigmented (white) onion, (C) leek, and (D) shallot.

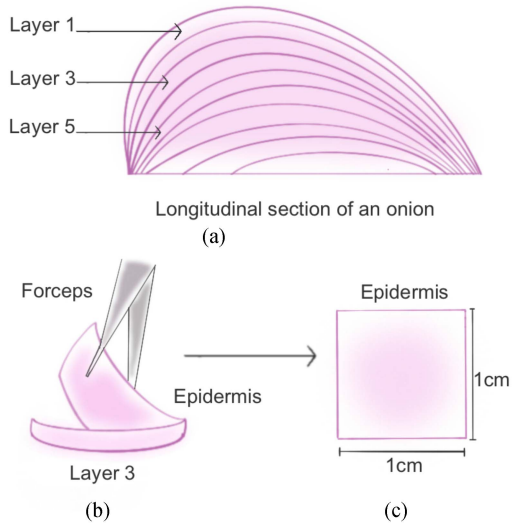

Fig. 3. (a) Schematic of a longitudinal section of an onion showing the various layers. (b) Schematic demonstrating epidermis extraction from Layer 3. (c) Schematic representation of 1 cm × 1 cm epidermis sample.

performed on these sections of extracted epidermis, which were mounted on an acrylic sample holder containing six 1 cm × 1 cm exposure windows and a metal backing plate (see Fig. 3). The sample holder was mounted onto the imaging stage and imaged continuously for 6 h in transflectance mode (see Fig. 4). On average, the ambient temperature varied between 22–24 °C, whereas corresponding relative humidity was 55%–65% over the 6 h measurement cycle.

In total, three separate imaging experiments were conducted in order to determine the following:

1) interaction between cell age and THz contrast;
2) orientation of the cells and its effect on THz contrast;
3) spectral differences between different species of the genus in the THz frequency.

The onions were selected by measuring their latitudinal and longitudinal diameters to ensure that the size variation was nominal. On average, it was observed that these onions had approximately ten layers between the first fleshy layer and the terminal bud. In experiment 1, the epidermis of pigmented and nonpigmented onion samples were extracted from three different depths, where the layer depth was indicative of cell maturity (i.e., outer layer contains older and more mature cells than the inner layers). The layers were extracted from out-to-inward direction by first removing all scaly leaf layers. The outermost first layer containing fleshy leaves was labeled as Layer 1. Successive samples from Layers 3 and 5 were extracted in relation to Layer 1 (see Fig. 3). In experiment 2, epidermis samples from pigmented onions, nonpigmented, onions, and leeks were all extracted from Layer 3 and then imaged in horizontal and vertical orientations. In experiment 3, epidermis samples of Layer 3 were extracted from pigmented onions, nonpigmented onions, leeks, and shallots. All samples in experiment 3 were measured in the same orientation. The selection of Layer 3 was based on microscopic analysis of each layer, where it was found that Layer 3 showed the least variation in cell size in the tissue sample.

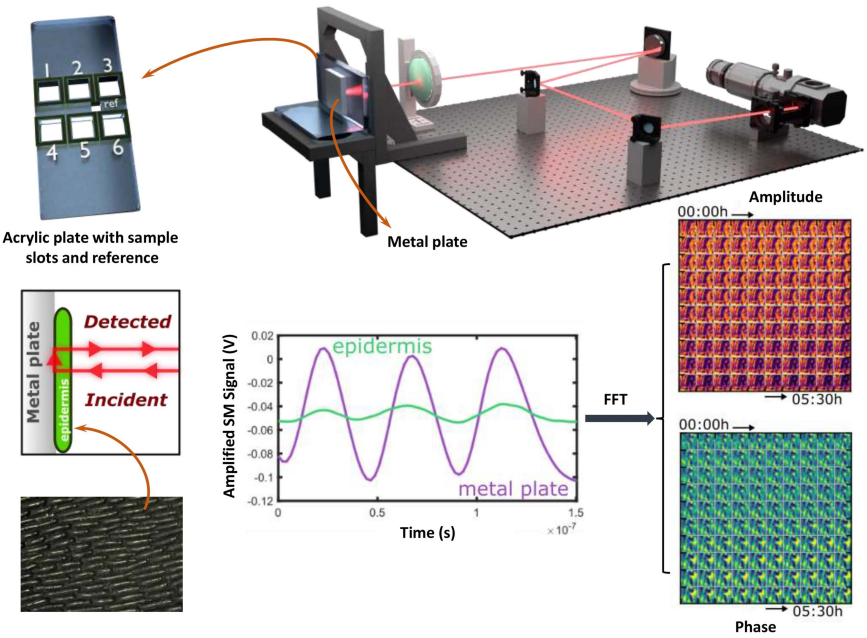

Fig. 4. (a) LFI system with samples mounted on the sample holder. (b) Acquired self-mixing signal from every sample and reference slot was amplified and fast Fourier transformed to generate amplitude and phase images for every frame of every slot.

TABLE I
SYSTEM PARAMETERS FOR LFI IMAGING SETUP

System parameters	THz –QCL–LFI
Frequency	2.8 THz with 600 MHz Freq. Sweep
Output power	2 mW peak
Image scan area	50 (H) × 40 (V) mm^2
Image pixel area	1000 (H) × 400 (V) (0.4 M pixels)
Pixel size	50 (H) × 100 (V) µm
Image acquisition time	2 min 40 Seconds (Mechanically Limited)

B. System Specifications

This study was carried out using an LFI imaging system, which is based on THz (see Table I) [42] with the technique comprising of a highly compact and highly sensitive system [49] where the emitter also acts as the detector [50], [51]. In this system, THz is generated by an encased QCL chip using a Stirling cooler at 50 K. The beam passes through a 30-mm collimating lens (Tsurupica, BBlaser Inc.), and follows a path through a set of 75-mm static reflecting mirrors, a 50-mm rasterizing focusing mirror (optics in motion), and a 50-mm focusing lens (Tsurupica, BBlaser Inc.) to the imaging mount before being reflected back to the QCL. This results in self-mixing of the interferometry signal, which is then amplified and Fourier transformed to generate its corresponding amplitude and phase values that allow for image formation [52]. As THz is highly sensitive to water, it is this dynamic response over time that can be detected and its pattern studied using the LFI system, which is known for its stability to optical feedback, high output power, and low phase noise [53]. The use of a QCL as both source and detector leads to background radiation suppression and increase detector sensitivity [54], [55].

C. Image Processing and Statistical Analysis

The acquired reflectance amplitude images were transformed to absorbance values by normalizing them with the reflectance of the metallic reference standard according to

$$A(x,y) = -20\log\left(\frac{R_{\text{onion}}(x,y)}{R_{\text{reference}}}\right) \tag{1}$$

where $A(x,y)$ is the absorbance at location x–y, $R_{\text{onion}}(x,y)$ is the reflectance of the onion layer affixed to the metallic backing plate, and $R_{\text{reference}}$ is the mean reflectance of the metallic reference standard (the backing plate with no sample attached to it). The acquired phase image values were wrapped between $-\pi$ and π and had to be unwrapped to reconstruct the underlying phase information. The unwrapped phase images were then normalized to the reference standard by subtracting the phase of the reference from the measured samples according to

$$\phi(x,y) = \phi_{\text{onion}}(x,y) - \phi_{\text{reference}} \tag{2}$$

where $\phi(x,y)$ is the normalized phase at location x–y, $\phi_{\text{onion}}(x,y)$ is the phase value of the onion sample at location x–y, and $\phi_{\text{reference}}$ is the mean phase value of the reference standard. Each measured frame was compressed to amplitude and phase contrast values in order to generate an *evaporation profile* for each sample. The contrast values were calculated as the standard deviation of all pixel values within frame. This variant of contrast is commonly used for digital images and is also known as the RMS contrast. The resulting time series (for both amplitude and phase values) were mean-subtracted and smoothed with a third-order Savitzky–Golay filter with a filter length of 11 frames (approx. 35 min) to better capture the long term trends of dehydration mechanisms.

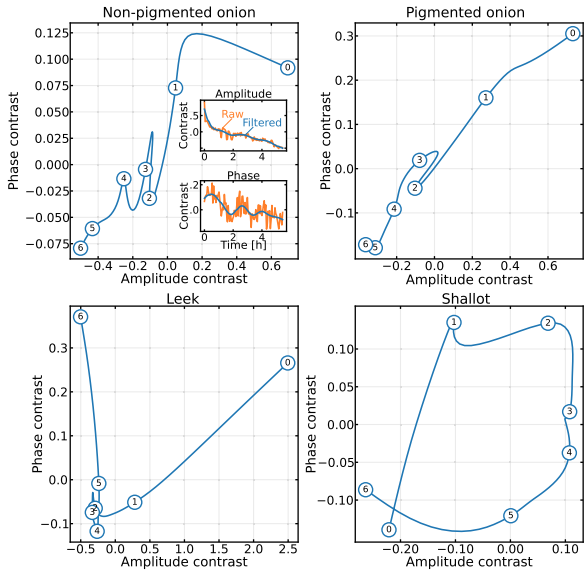

Fig. 5. Evaporation profile of nonpigmented onion, pigmented onion, shallot, and leek showing the phase and amplitude correlation over the period of 6 h.

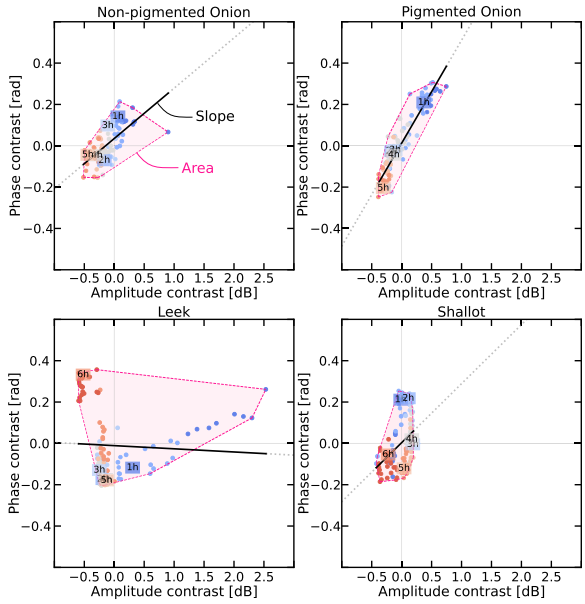

Fig. 6. Evaporation profiles of nonpigmented onion, pigmented onion, shallot, and leek over the period of 6 h show their unique and repeatable slopes and area proving that same morphology can form distinct patterns that could be attributed on their biochemical makeup and percentage water to cell volume ratio.

In order to compare evaporation profiles between different sample types and cell orientations, each profile was cutoff at 6 h and described using the area under the profile and angle of the amplitude–phase cluster. The area of the profile was determined by first encapsulating it inside a 2D convex hull and then calculating the area of the hull was extracted with a first-order polynomial approximation.

IV. Results and Discussion

The evaporation profile of each type of *Allium* sample for a 6-h time period is depicted in Fig. 5. Notably, the most significant changes occurred within the first 6 h, during which the thin sample layers undergo dehydration [56]. This time frame also allowed us to account for atmospheric fluctuations and system stability, in addition to the sample dehydration process, allowing for a detailed examination of the critical stage. Detailed comparison for all the four types of *Allium* samples based on the area encompassed by the amplitude–phase shift and the trajectory taken in the duration of the experiment is shown in Fig. 6. As the graphs suggest, it is not only the size of the cells that attribute to the difference in the trend, but also the biochemistry of the biomolecules enclosed in these cells. Both pigmented and nonpigmented onions have comparable cells sizes (500 μm $\times$ 50 μm), whereas shallots and leeks have comparable cell sizes (700 μm $\times$ 30 μm). Hence, any variation between them could be accredited to the difference in biomolecules. This observation is consistent with the fact that water interaction inside the cell is primarily determined by its contents [57], [58]. Our initial analysis suggests that images taken from same type of cells, from corresponding positions of the plant, and with similar cellular dimensions will exhibit different time-dependent THz contrasts. This would be determined by the biochemistry of the cells and how water interacts with the biomolecules over time.

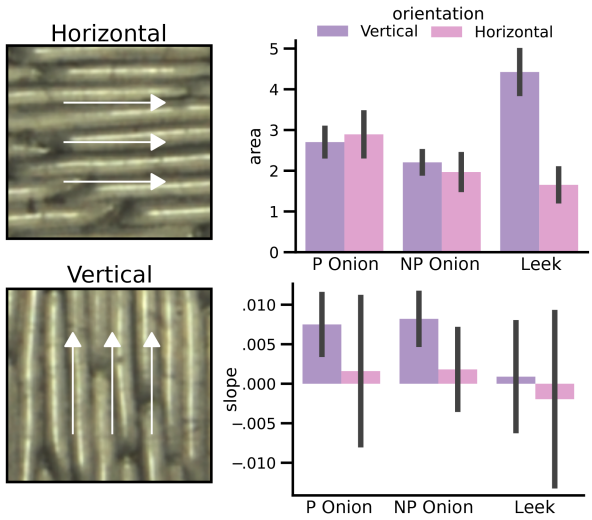

Fig. 7. Comparison of the slope and area between pigmented onions, nonpigmented onions, and leeks determined that cellular orientation would not lead to data variability in the case of onions. However, due to the composite nature of the leek epidermis layer, data variability can be observed between cells in vertical and horizontal cell orientations.

The difference in water evaporation trajectory and spread between *Allium* types and experimental conditions were summarized by extracting the linear slope and area under the curve inside of the amplitude–phase domain for each sample (see Fig. 6). Comparison of these evaporation parameters revealed differences in plant types and cell maturity but not in cell orientation apart from leek (see Fig. 7).

This study investigated the evaporation mechanisms of different layers of *Allium* plants using THz imaging. Layers 1 and

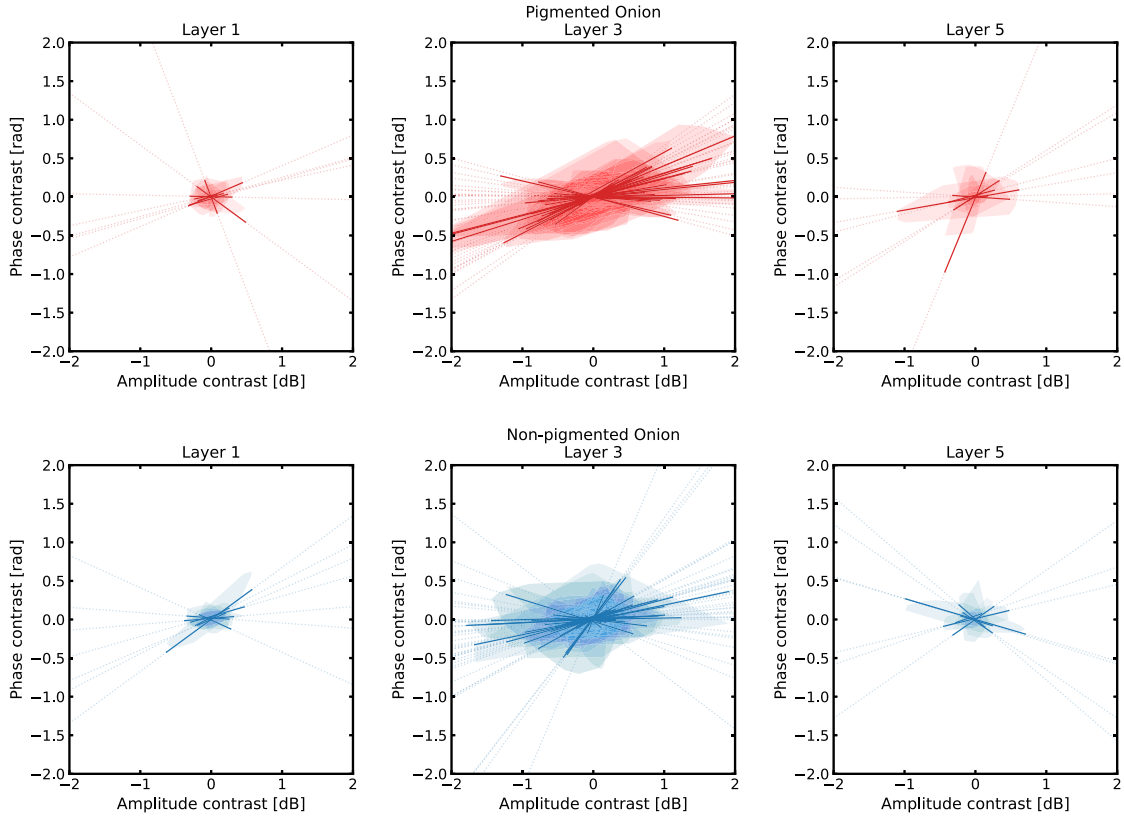

Fig. 8. Comparison of the spread and trajectory between layers 1, 3, and 5 in pigmented and nonpigmented onions showing the variability range between cell sizes. Each subfigure shows the distribution in amplitude–phase space of the 135 datapoints obtained from measurements performed over 6 h on a given layer 1, 3, or 5 of a sample [as explained in Fig.3(a)]. The lines correspond to the linear fit through the amplitude–phase data points of each sample. The solid lines represent the linear fit near the datapoints while dashed lines are the extrapolation outside the datapoints.

5 were measured seven times each, whereas Layer 3 with the most consistent cells was measured 40 times (see Fig. 8). Here, we compared the results for Layers 1, 3, and 5 of pigmented (top row) and nonpigmented (bottom row) onions showing the variability range between cell sizes. Results indicate that the amplitude–phase spread (area enclosed) over the duration of the experiment was larger for Layer 3 as compared with Layer 1 (cells approaching senescence) and Layer 5 (juvenile cells). While juvenile cells offered efficient water retention, their significant size variation rendered them unsuitable for our experiment. In comparison, mature cells have smaller cell size variation and offer effective water retention. However, cells approaching senescence lead to lower permeability, vacuole shrinkage, and thicker cell walls, which reduces their ability to store water. This helped determine that Layer 3 would be best suited for experimental purpose due to its dynamic nature over the duration of the experiment as we could observe better species specific correlation of time dependent evaporation response from these mature cells [7].

All used samples were extracted from different species of the same genus. Since all members are genetically related, we investigated the ability of THz imaging and evaporation profiles to differentiate between the species of a single genus (see Fig. 9). Again, the main differences in their evaporation profile parameters were observed in the area under the curve, where the area occupied by leek samples was on average much greater than the areas of the other three species. We hypothesize that the observed effect is a result of cellular variability that, in turn, arises from genetic variability. We also saw pronounced variation in the trajectory between leek and shallot, which could point to the presence of stomata and guard cells present on the epidermis. In case of the pigmented and nonpigmented onions, the slight variation in the spread could be attributed to the fact that the only thing that distinguishes them is the presence or absence of flavonoids. The proposed technique could thus potentially be used as a fingerprint to identify between genetic variants.

Cellular arrangement of *Allium* genus is not isometric, meaning that the cells are always organized in specific parallel configuration. In order to test whether evaporation profiles are rotation-invariant with respect to this structure, two sets of horizontal versus vertical measurements were performed on samples obtained from the third layer. This measurement was repeated for pigmented onions, nonpigmented onions, and leeks (see Fig. 7). No substantial differences were observed between the two orientations for pigmented onions and nonpigmented onions. The lack of significant differences could be attributed to the fact that the individual cells are too small to influence the resulting THz image and the main contrast is generated by the bulk of the tissue. However, there was significant difference between the horizontal and vertical orientation in leek. This

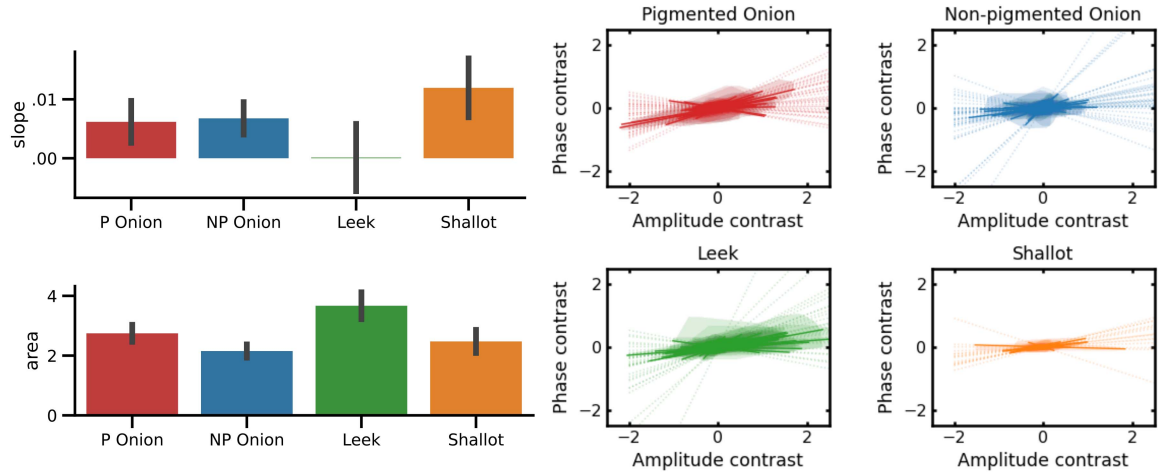

Fig. 9. Comparison of the slope and area as extrapolated from the trajectory and spread between pigmented onions, nonpigmented onions, leeks, and shallots showing the presence of unique fingerprints to identify between genetic variants.

could be due to the presence of guard cells and stomata in the bulk tissue thereby interrupting its regular arrangement.

Shifts in amplitude and phase contrasts of the THz images were observed under nearly all experimental conditions of the experiment. The most likely cause for these shifts is the water transfer occurring inside the cellular matrix as evaporation drives the cells toward plasmolysis. This time-dependent evaporation is guided by several factors, such as the size of the cells and changes in the composition of biomolecules [56], [59], [60], [61]. As these factors vary between different species of the *Allium* genus, it is, therefore, reasonable to employ the species specific evaporation profile as measured by the rapid method of THz-LFI imaging to identify genetic variants.

V. Conclusion

This study investigates the application of THz spectroscopy for real-time monitoring of unique biochemical and biophysical interactions during progressive dehydration in a model plant cell system—the epidermal cell in its tissue form. The age of the samples was optimized to minimize variations in their planar spread, a crucial parameter for consistent data acquisition. Furthermore, the presence of a heterogeneous monolayer was identified as a source of data variation due to cell orientation. By controlling these two factors, the study successfully distinguished between members of the *Allium* genus based on unique trajectories and two dimensional distributions in amplitude-phase space obtained through THz spectroscopy.

The high genetic similarity within a genus makes the *Allium* example particularly compelling. This work establishes a foundation for employing THz spectroscopy to detect subtle genetic variations between plant species by analyzing the biochemical and biomechanical fingerprints observable in the THz range and arising from water interactions within their tissues.

Our technique was able to effectively and efficiently detect variation in the dehydration profiles of members of the same genus. We can conclude that mature cells, regardless of tissue orientation or size, will exhibit this variability based on their biochemical composition, which can be mapped by the THz–QCL–LFI. The dehydration profiles were acquired by encapsulating the time-dependent evaporation trajectory in the amplitude–phase space with a convex hull. The slope and area under the hull can be used to discriminate between different members of the *Allium* family. The proposed techniques leverage the inherent sensitivity of THz radiation toward water and the natural variation in evaporation mechanisms between different plant species. It is known that a GM cell will show variation from its wild type by the virtue of its size, presence or absence, and or overexpression of certain significant biomolecules [62], [63]. This technique could potentially be used for rapid sorting of crops based on the presence and/or absence of specific genetical components. As modern agriculture is constantly developing toward mitigating risks for food security and facilitating genome conservation, a robust nondestructive image-based identification method for detecting different genetic variants could be especially useful.

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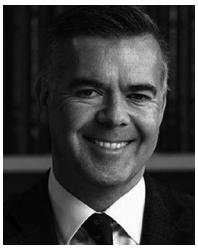

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