
Proceedings Paper:
Williams, J., Lin, W.C., Li, W. et al. (3 more authors) (2020) Translating optical coherence tomography technologies from clinical studies to botany : real time imaging of long-distance signaling in plants. In: Proceedings, Biophotonics Congress: Biomedical Optics 2020 (Translational, Microscopy, OCT, OTS, BRAIN). Optical Coherence Tomography, 20-23 Apr 2020, Washington, DC, USA. OSA Publishing . ISBN 9781943580743

https://doi.org/10.1364/oct.2020.om4e.6

Translating Optical Coherence Tomography Technologies from Clinical Studies to Botany: Real Time Imaging of Long-Distance Signaling in Plants

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Abstract: The time has now come to expand the use of optical coherence tomography and apply it in botany where the technology's key advantages enable visualization of plant's communication as it was never done before.

1. Introduction

Optical coherence tomography (OCT) has evolved to become a standard for medical diagnosis such as in ophthalmology, oncology and dermatology. Indeed, because of its non-ionizing scanning probe and high-speed data acquisition, it allows for real time, non-destructive, non-invasive *in vivo* studies of living tissues. Given the success of OCT in clinical studies, the goal is to exploit the technique's key advantages and apply it to another field of natural science: botany. Accordingly, in this study, we demonstrate how OCT can be used to study long-distance communication in plants.

Given the fact that plants cannot escape environmental changes, nor pathogen or herbivore attacks, they require efficient mechanisms to recognize the type of stressor, convey the information and respond in a targeted manner. Plants are thus able to activate hydraulic, chemical and/or chemo-electrical long-distance signals to initiate systemic responses to the aggression [1]. Following the "squeeze cell hypothesis" [2], an inflicted wound induces fast changes in water pressure along the xylem (i.e. one of the plant's main vein), which, in turn, lead to significant changes in turgor pressure in adjacent cells. The rapid pressure changes in these adjacent cells are expected to activate ion and water fluxes, which directly or indirectly controls the synthesis of jasmonates. Jasmonates are critical for the plant defense system as they regulate gene expression of various chemicals that either limit the spread of pathogens or debilitates herbivores. The production of jasmonates is thus linked to variation in cell-pressure and ion concentration. However, our knowledge about the mechanism that triggers the production and liberation of jasmonates is hindered by practical constraints: ideally, we require a non-invasive tool to monitor these mechanisms *in-vivo* and live.

In order to verify this hypothesis and to further investigate the plant's communication and defense mechanisms, we use OCT to monitor the morphological changes of the plants that are induced by an external stimulus. As mentioned, OCT ideally allows for non-invasive and live imaging of the plant's inner structure. Furthermore, the technique is fit to operate at room temperature, in normal atmosphere and under normal lighting conditions. It is thus appropriate to the natural environment of the plant.

2. Material and Methods

The OCT equipment is set to acquire ~50 A-scans/min across the central vein (xylem and phloem) of a young tomato leaf (see Fig. 1. a-d). The stimulus is a 30 seconds-long, 800 nm, 6 W laser pulse focused on the central vein of an adjacent leaf (see Fig. 1. c). The motion of the leaf is tracked by monitoring the A-scans difference images, which are obtained by subtracting each A-scan with a reference A-scan (as illustrated in Fig 1. f). In such difference image, the motion of the leaf is represented by changes in grey tones: the cells move from darker region to lighter regions. The grey tones being coded (i.e. black = -0.5, white = +0.5), the overall motion to the leaf is computed by integrating the pixel value of each A-scans difference images. Following this procedure, a simple translation and rotation of a cell results in positive and negative region of similar amplitude, which will cancel each other while computing a normal integral (i.e. straight-forward summation of all pixel values, as seen in Fig. 1. e, red curve). Such integral thus track any cell motions that results in non-symmetric changes such as expansion and shrinkage. To monitor the overall motion of the cells, including simple translations and rotations, a second integral is performed, where the pixel values of the difference images are first squared, and the sum is subsequently square-rooted (as seen

in Fig. 1. e, black curve). Consequently, any features present in the black curve that are not correlated to features in the red curve correspond to whole leaf displacements. Note that the light scattering of the wounding laser is also picked up by the OCT probe and results in an increased value in both integrals (illustrated by the artifact in Fig. 1 e, between 0 and 0.5 min).

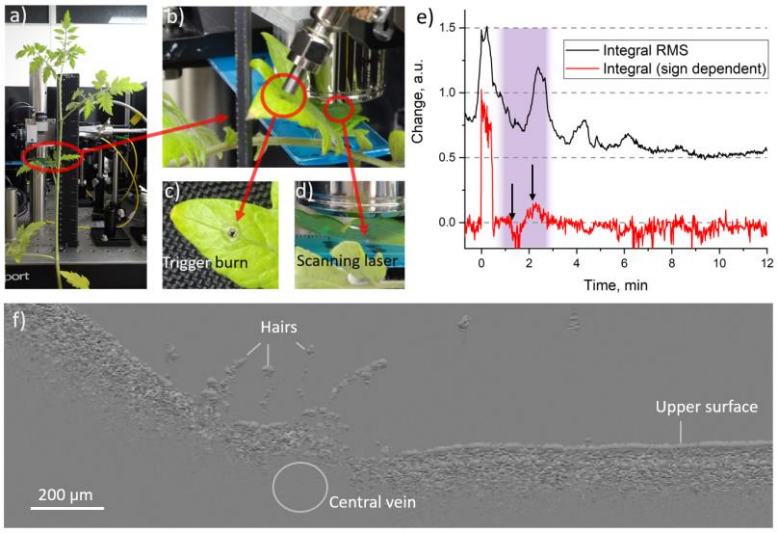

Fig. 1. (a-b) Experimental setup with (c) an example of laser burn used as stimuli while (d) A-scanning the cross-section of an adjacent tomato leaf with OCT. (e) Overall motion of the leaf represented as the time-dependent integral of the A-scans difference image. Red curve is the sign-dependent integral (normalized). Black curve is the root-integral-squared signal magnitude (normalized and vertically shifted for clarity). The feature at 0-0.5 min is partially due to light-scattering from the 30 second-long wounding laser pulse. The purple shaded area is the expected slow response of the adjacent leaf. (f) A-scan difference image obtained by subtracting A-scans taken at times indicated by the black arrows in panel (e). The grey background signifies no motions. Darker and lighter colors correspond to negative and positive pixel values, respectively. Thus, the plant's cells moved from a darker region toward a lighter region.

3. Results and Discussion

Upon wounding, the leaf undergoes an immediate jerk, which relaxes when the wounding laser switches off. Similar to a reaction coordinate in quantum mechanics, the integrals presented in Fig. 1. e, shows the overall leaf changes. This spasm-like reaction is illustrated by the initial signal (shown in Fig. 1. e, black curve) in the region from 0 to 1.0 min. This instantaneous reaction is certainly due to a fast hydraulic signal in response to the local increase in temperature caused by the wounding laser. Once perforated, the leaf is expected to generate a type of self-sustained action-potential that travels through the whole plants [3]. Following the squeeze-cell hypothesis [2], the action potential-like signal is characterized by fast changes in water pressure along the xylem, which, in turn, lead to significant changes in turgor pressure in adjacent cells. It is these slower changes in water pressure that are expected to affect the morphology of the near-by cells and overall leaf shape. The data suggest that the leaf did react ~2 min after wounding with a displacement (Fig. 1. e, black curve) as well as with changes in density (Fig. 1. e, red curve). In order to pin-point the exact type and location of morphological changes that is taking place, more specific image processing tools are currently being investigated, such as registration algorithms.

In conclusion, it is important to mention that the present data is the first in-vivo, label-free and non-invasive monitoring of a plant's inner cellular structure! Enabled by the use of OCT technology, this initial study is thus the first of its kind in the promising field of plant signaling.

4. References

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