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3D visualization of plant-pathogen interaction inside plant leaves with dynamic contrast optical coherence tomography

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Abstract: Optical coherence tomography (OCT) can image deep inside scattering plant tissue with micrometer resolution. However, conventional OCT lacks specificity to distinguish plant tissue from pathogen tissue. Here we show how dynamic OCT (dOCT) creates functional contrast of Bremia, a downy mildew in lettuce, based on sub-resolution dynamic activity inside the tissue. We demonstrate its applicability for disease resistance quantification and longitudinal study of pathogen growth.

1. Introduction

Plant resistance against pathogens is an important trait that growers require to efficiently grow crops. To develop plants with resistance against pathogens, it is essential to understand the interaction between plant and pathogen. Imaging plays a pivotal role in visualizing plant-pathogen interaction, the local plant resistance response, and provides a way to perform this in a high-throughput way.

Optical coherence tomography (OCT) is a label-free imaging method that can image deep into plant tissue at resolutions of pathogen structures, especially when combined with water-infiltration of the plant leaf [1]. However, with conventional OCT, the scattering contrast is not sufficient to distinguish between plant and pathogen tissue. Dynamic OCT (dOCT) uses the power distribution of the temporal fluctuations in the OCT amplitude of the scattered signal to create functional contrast at pixel level [2]. Since, plant cells and pathogens have different subcellular activity, dOCT can create sufficient contrast. Here, we show dOCT non-invasive label-free 3D imaging of *Bremia* in plant leaves. *Bremia* is a pathogen that grows in plant leaves and has thin, root-like, structures that are called hyphae. Whereas conventional techniques are destructive, label free dOCT method can improve imaging throughput, enable longitudinal 3D visualization of plant-pathogen interaction, and quantify local plant resistance.

2. Methods

Figure 1 shows dOCT imaging applied to plant leaves. Repeated B-scans are obtained at the same location. The OCT amplitude in time is Fourier transformed. The mean intensity for each frequency band is log compressed and transformed to an RGB color value. The frequencies of the fluctuations reveal information of the time scale of the microscopic, sub-resolution motion of the tissue. The first frequency band is the mean pixel value (blue). The second frequency band, with intermediate frequencies between 1 and 5 Hz gives strong contrast for *Bremia*, but also for leaf veins and plant cell walls (green). The third, frequency band ranges from 5 to 20 Hz(red).

The measurement are performed with a commercial SD-OCT system (Ganymede II HR, Thorlabs) [1]. A high resolution scan lens (OCT-LK2-BB, Thorlabs) was used with a lateral resolution of about 3 µm (FWHM), close to the axial resolution of 2.2 µm in tissue. A leaf disc was punched out of a lettuce leaf that was infected with *Bremia* 7 days prior to imaging. The leaf disc was water infiltrated by placing it in a syringe with water. An enface area of 1.08×0.45 mm^2 was in 3D by dOCT.

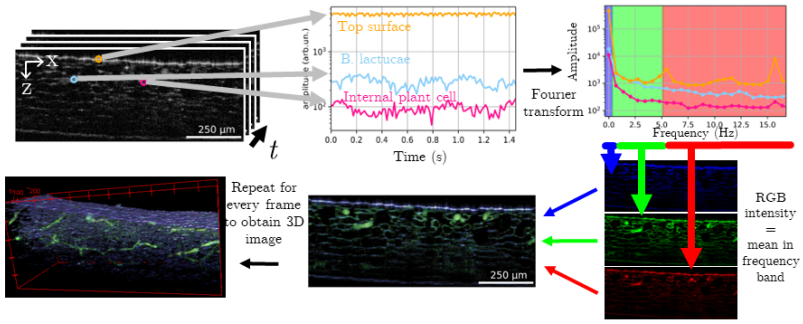

Fig. 1. Schematic overview of dynamic OCT applied to plant leaves.

3. Results and discussion

To evaluate the value of 3D label-free plant pathogen imaging for digital phenotyping, we quantified the infection level in the leaf tissue different lettuce varieties to downy mildew, see Fig. 2(a). Imaging was performed on a total of 48 volumetric leaf segments. Quantitative phenotyping was performed by investigating the presence, the volume, and length of the *Bremia* hyphae in each of the 48 3D dOCT images, see Fig. 2(b). Variety Bedford had 4/16 volumes colonized, with an infection volume below 0.2 nl/mm^2 and length below 3 mm/mm^2. Variety Iceberg had 6/16 volumes colonized, 50% more than Bedford with an infection volume up to 0.6 nl/mm^2 and length up to 8 mm/mm^2. In contrast, variety Salinas had 15/16 colonized volumes with an infection volume reaching up to 2.3 nL/mm^2 and length reaching up to 18 mm/mm^2.

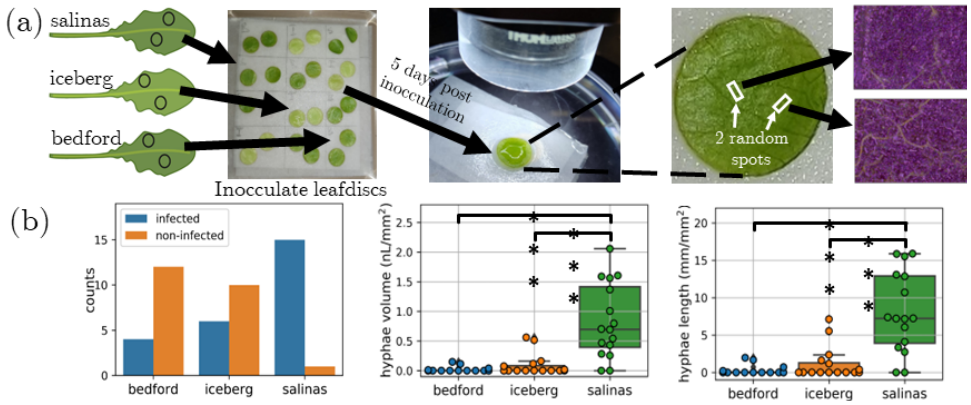

Fig. 2. (a) dOCT plant-pathogen phenotyping. (b) Quantification of infection by hyphae presence, volume and length.

We demonstrate quantitative assessment of temporal development of *Bremia* by in vivo 3D imaging of infected Salinas leaf discs. dOCT measurements visualize the development of the 3D *Bremia* hyphal network inside lettuce leaf tissue, see Fig. 3(a). From these dOCT measurements the time of initial growth of individual segmented hyphae are visualized in 3D, Fig. 3(b), and the length of individual hyphae quantified in time, Fig. 3(c).

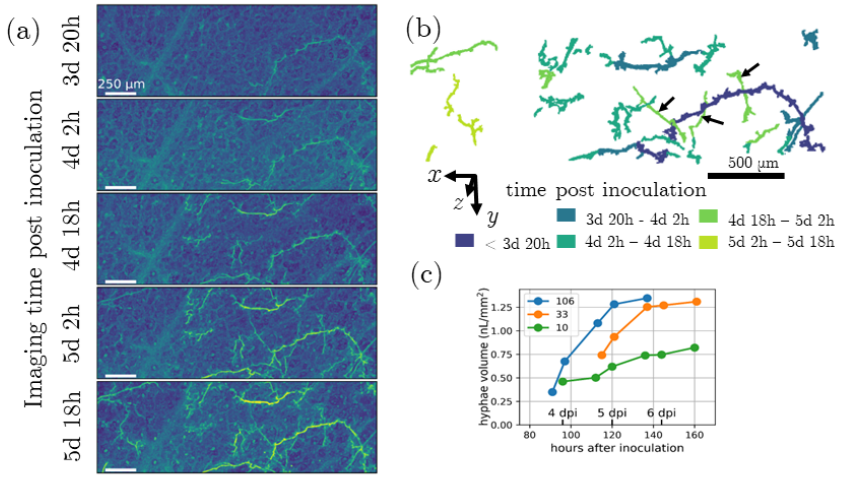

Fig. 3. (a) *Bremia* hyphae development over time imaged with dOCT contrast. (b) Growth pattern visualization. (c) Hyphae length over time.

Our work shows that we clearly can reveal plant pathogen structures in in vivo plant tissue that cannot be visualized in vivo with conventional (OCT) imaging. This opens us a range of opportunities for plant scientists to study in vivo plant-pathogen interaction without the need for (fluorescent) labeling.

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