On-field optical imaging data for the pre-identification and estimation of leaf deformities

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Visually nonidentifiable pathological symptoms at an early stage are a major limitation in agricultural plantations. Thickness reduction in palisade parenchyma (PP) and spongy parenchyma (SP) layers is one of the most common symptoms that occur at the early stage of leaf diseases, particularly in apple and persimmon. To visualize variations in PP and SP thickness, we used optical coherence tomography (OCT)-based imaging and analyzed the acquired datasets to determine the threshold parameters for pre-identifying and estimating persimmon and apple leaf abnormalities using an intensity-based depth profiling algorithm. The algorithm identified morphological differences between healthy, apparently-healthy, and infected leaves by applying a threshold in depth profiling to classify them. The qualitative and quantitative results revealed changes and abnormalities in leaf morphology in addition to disease incubation in both apple and persimmon leaves. These can be used to examine how initial symptoms are influenced by disease growth. Thus, these datasets confirm the significance of OCT in identifying disease symptoms nondestructively and providing a benchmark dataset to the agriculture community for future reference.

Background & Summary

Plant and fruit diseases are impairments of the normal state of a plant that interrupt or modify its vital functions. Apple is one of the most widely produced fruits globally, whereas persimmon is mainly cultivated in East Asian countries, such as Korea, Japan, and China1,2. Apple scab, marssonina leaf blotch, black rot canker, and alternaria leaf spot/blight are examples of diseases of apple, in which symptoms can be identified on the leaves after disease progression3–5, which reduce the quantity and quality of the produce. Circular leaf spot is the most damaging pathogenic disease in persimmon cultivation6, causing discoloration and defoliation of diseased leaves and resulting in massive economic losses7,8. In most cases, the disease can be treated and controlled if the symptoms are identified at an early stage9,10.

The initial symptoms of these diseases occur mainly on the leaf subsurface, which is a complex organ comprised mostly of palisade parenchyma (PP) and spongy parenchyma (SP), crossed by vascular tissue, and surrounded by two epidermises11. The PP has regular-shaped cells near the upper surface of the leaf, whereas the SP is less well-organized and located near the lower epidermis of the leaf12,13. These layers are crucial in manufacturing food, gas exchange, and water evaporation. Therefore, the pre-identification of plant diseases by detecting abnormalities of PP and SP is important for appropriate timing control by reducing damage and production cost and increasing production. The schematic shown in Fig. 1 elaborates the inner morphology of leaf specimens illustrating gradual structural changes and thickness reduction with disease progression between PP and SP.

Several methods have been introduced for the early detection of leaf diseases. Visual inspection is commonly used; however, it is subjective, inefficient, time-consuming, labor-intensive, and costly in the early stages of infection14–16. In contrast, physiological, biological, serological, and molecular tests are laboratory-based

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methods used for the identification of plant disease\textsuperscript{17–20}. Polymerase chain reaction, enzyme-linked immunosorbent assay, and histological sectioning are some laboratory test-based plant disease evaluation methods that are destructive, complex, time-consuming, and expensive\textsuperscript{21,22}. To compensate for these drawbacks in plant disease detection, noninvasive techniques, such as image processing\textsuperscript{23–26}, terrestrial laser scanning\textsuperscript{16}, sonic tomography\textsuperscript{18}, electronic nose\textsuperscript{27}, microfocus X-ray fluorescence\textsuperscript{28}, GanoSken technology\textsuperscript{29}, and spectroscopy\textsuperscript{30}, have gained much attention. However, a long setup process, complexity, high cost, sensitivity to environmental change, low selectivity, and highly specific software requirements\textsuperscript{20,31} are some drawbacks of these techniques. Noninvasive morphological and structural imaging of plant materials has been performed using ultrasound\textsuperscript{32}, X-ray\textsuperscript{33}, magnetic resonance imaging\textsuperscript{34}, and positron emission tomography imaging\textsuperscript{35}. However, these imaging techniques have low image resolution and long image acquisition time\textsuperscript{36–39}. Therefore, a noninvasive optical imaging technique is required for the early detection of plant disease progression by investigating subsurface structures of leaf specimens.

High-resolution optical coherence tomography (OCT) is a noninvasive imaging technique that provides cross-sectional images using a nonionizing broadband light source\textsuperscript{40}. The image resolution of OCT is 1–15 μm (10–100 times better than ultrasound)\textsuperscript{41}. OCT has been widely used in various fields, including medical diagnosis\textsuperscript{42,43}, dentistry\textsuperscript{44,45}, dermatology\textsuperscript{46,47}, tissue imaging\textsuperscript{48,49}, agriculture\textsuperscript{50,51}, and industrial applications\textsuperscript{52,53}. Because OCT imaging depth (1.5–2 mm) is suitable for the micrometer-scale visualization of the internal structure of plant leaves, OCT-based agricultural disease detection studies have established a solid platform to confirm the applicability of OCT in plant disease inspection\textsuperscript{54–62}.  

![Fig. 1](https://example.com/fig1.png) **Fig. 1** A schematical illustration of leaf inner morphology and its gradual changes from healthy to infected state.

![Fig. 2](https://example.com/fig2.png) **Fig. 2** Schematic of the spectral-domain optical coherence tomography (SD-OCT) system used for data acquisition. CB: capture button; GS: galvo-scanner; L: lens; LCD: liquid crystal display.
To elaborate the potential merits of OCT for inspecting plant diseases, various coordinates of OCT images and an optical signal intensity-based depth profile algorithm are presented in this study. The developed algorithm was incorporated to set a threshold for the pre-identification of PP and SP layer abnormalities in persimmon and apple leaf specimens by assessing OCT cross-sectional images. The developed depth profile algorithm was applied to cross-sectional OCT images to quantitatively evaluate the inner layer structure of leaf specimens. The obtained data sets revealed a gradual thickness reduction between PP and SP layers in healthy,
Table 1. The disease name and the causative agents of the persimmon and apple leaves.

| Persimmon | Apple |
|-----------|-------|
| Disease name | Circular leaf spot | Apple blotch |
| Causal agent | Mycosphaerella nawae | Diplocarpon coronariae |

Fig. 5 Width and height measurement algorithm of apple leaf layer intensity peaks. (a) 2D cross-sectional image of apple leaf with different layers. (b) 2D cross-sectional image after flattening. (c) Depth profiles of three regions of interest (ROIs) of a single leaf. (d) Average depth profile of three ROIs of a single leaf. (e) Width and height measurement of leaf layer intensity peaks. UE: upper epidermis, PP: palisade parenchyma, SP: spongy parenchyma.

Fig. 6 2D cross-sectional images of healthy, apparently-healthy, and infected persimmon and apple leaves. (a–c) 2D cross-sectional images of healthy, apparently-healthy, and infected persimmon leaves, respectively. (d–f) 2D cross-sectional images of healthy, apparently-healthy, and infected apple leaves, respectively. UE: upper epidermis, PP: palisade parenchyma, SP: spongy parenchyma.
Fig. 7  Persimmon and apple leaf layer thickness. (a) Scatter plot of the layer thickness of healthy, apparently-healthy, and infected persimmon leaves. (b) Comparison of layer thickness of persimmon leaves. (c) Scatter plot of the layer thickness of healthy, apparently-healthy, and infected apple leaves. (b) Comparison of layer thickness of apple leaves.

Fig. 8  Scatter plot of upper epidermis (UE), palisade parenchyma (PP), and spongy parenchyma (SP) layer peak width and height of healthy, apparently-healthy, and infected apple leaf intensity. (a) Intensity peak height/normalized intensity of the UE layer peak of healthy, apparently-healthy, and infected apple leaves. (b) Intensity height of the PP layer peak of apple leaves. (c) Intensity height of the SP peak of apple leaves. (d) UE layer peak width of healthy, apparently-healthy, and infected apple leaves. (e) PP layer peak width of apple leaves. (f) SP layer peak width of apple leaves.
apparently-healthy, and infected specimens of persimmon and apple leaves. A threshold value was set based on the thickness differences obtained from the collected datasets for detecting early abnormalities in persimmon and apple leaves, which can be used to assess plant leaf diseases in the future.

Methods

Optical imaging modality. The schematic of the optical configuration of SD-OCT used in this study is shown in Fig. 2. The system was equipped with a broadband light source (EXS210068-01, Exalos, Switzerland) with a central wavelength of 850 nm, full width at half maximum bandwidth of 55 nm, and average output power of 5 mW. A galvanometer-based optical scanner (GVS002, Thorlabs, USA) and a 1-inch object lens (AC254-030-B, Thorlabs, USA) were used for scanning the samples.

Table 2. Persimmon leaves layer thicknesses.

![Table 2](image_url)

Fig. 9 Comparison of average height and width of intensity peaks of healthy, apparently-healthy, and infected apple leaves. (a) Comparison of the intensity peak heights of healthy, apparently-healthy, and infected apple leaves. (b) Comparison of the intensity peak widths of healthy, apparently-healthy, and infected apple leaves.
Thorlabs, USA) were used in the handheld probe-based sample arm for transversely scanning the sample. The reference arm was identically composed of a collimator (F260APC-B, Thorlabs, USA), lens (AC254-030-B, Thorlabs, USA), and mirror (PF10-03-P01, Thorlabs, USA). The ratio of the fiber couplers (TW850R5A2, Thorlabs, USA) used in this system was 50:50. The back-scattered signals from the sample and reference arms were coupled together through the coupler and transferred to a customized spectrometer. The spectrometer was calibrated using a previously described method.40 A 2048-pixel line scan camera (spL2048-140 km, Basler, Germany) was used for image acquisition. A miniature LCD panel was connected to the handheld scanner for real-time OCT image preview, and a 1.5 m handheld probe with a capturing button was connected to the handheld scanner to save the OCT image. The axial and lateral resolutions of this OCT system were 5.1 and 11 μm, respectively, when measured in air.

Plant leaf specimens. The photographs of persimmon and apple leaf specimens are presented in Fig. 3. Healthy, apparently-healthy, and infected leaves of the persimmon tree are shown in Fig. 3(a–c), respectively. Figure 3(d–f) represent healthy, apparently-healthy, and infected leaves of the apple tree, respectively. The healthy and apparently-healthy leaves of both persimmon and apple trees have a similar appearance; however, the infected leaves of both trees were discolored. The visual inspection method was unable to provide early detection of leaf abnormalities, whereas the analysis of the internal structure of healthy, apparently-healthy, and infected leaves of persimmon and apple trees could be performed by assessing OCT cross-sectional images.

| Healthy | Thickness (μm) | Leaf # | Thickness (μm) | Leaf # | Thickness (μm) | Leaf # | Thickness (μm) | Leaf # | Thickness (μm) | Leaf # | Thickness (μm) | Leaf # |
|---------|----------------|-------|----------------|-------|----------------|-------|----------------|-------|----------------|-------|----------------|-------|
| 1       | 55             | 23    | 57.5           | 45    | 67.5           | 67    | 62.5           | 89    | 60             | 111   | 70             | 113   |
| 2       | 52.5           | 24    | 65             | 46    | 70             | 68    | 75             | 90    | 70             | 112   | 72.5           | 134   |
| 3       | 52.5           | 25    | 57.5           | 47    | 62.5           | 69    | 70             | 91    | 65             | 113   | 75             | 135   |
| 4       | 85             | 26    | 55             | 48    | 70             | 70    | 7.5            | 92    | 62.5           | 114   | 77.5           | 136   |
| 5       | 62.5           | 27    | 62.5           | 49    | 60             | 71    | 6.5            | 93    | 67.5           | 115   | 72.5           | 137   |
| 6       | 50             | 28    | 49.7           | 50    | 70             | 72    | 67.5           | 94    | 75             | 116   | 72.5           | 138   |
| 7       | 55             | 29    | 62.5           | 51    | 55             | 73    | 67.5           | 95    | 77.5           | 117   | 75             | 139   |
| 8       | 65             | 30    | 75             | 52    | 67.5           | 74    | 72.5           | 96    | 75             | 118   | 75             | 140   |
| 9       | 50             | 31    | 52.5           | 53    | 57.5           | 75    | 70             | 97    | 70             | 119   | 72.5           | 141   |
| 10      | 57.5           | 32    | 57.5           | 54    | 65             | 76    | 62.5           | 98    | 77.5           | 120   | 65             | 142   |
| 11      | 50             | 33    | 60             | 55    | 65             | 77    | 62.5           | 99    | 62.5           | 121   | 67.5           | 143   |
| 12      | 65             | 34    | 54.5           | 56    | 62.5           | 78    | 72.5           | 100   | 65             | 122   | 70             | 144   |
| 13      | 50             | 35    | 54.5           | 57    | 65             | 79    | 75             | 101   | 57.5           | 123   | 85             | 145   |
| 14      | 52.5           | 36    | 50             | 58    | 65             | 80    | 75             | 102   | 57.5           | 124   | 75             | 146   |
| 15      | 50             | 37    | 62.5           | 59    | 72.5           | 81    | 65             | 103   | 65             | 125   | 75             | 147   |
| 16      | 52.5           | 38    | 57.5           | 60    | 67.5           | 82    | 67.5           | 104   | 60             | 126   | 60             | 148   |
| 17      | 60             | 39    | 60             | 61    | 62.5           | 83    | 72.5           | 105   | 80             | 127   | 67.5           | 149   |
| 18      | 55             | 40    | 62.5           | 62    | 65             | 84    | 75             | 106   | 75             | 128   | 60             | 150   |
| 19      | 55             | 41    | 65             | 63    | 67.5           | 85    | 75             | 107   | 72.5           | 129   | 75             | 151   |
| 20      | 55             | 42    | 67.5           | 64    | 65             | 86    | 75             | 108   | 65             | 130   | 75             | 152   |
| 21      | 67.5           | 43    | 72.5           | 65    | 70             | 87    | 67.5           | 109   | 70             | 131   | 70             | 153   |
| 22      | 57.5           | 44    | 65             | 66    | 70             | 88    | 65             | 110   | 70             | 132   | 77.5           | 154   |

Table 3. Apple leaves layer thicknesses.
### Table 4. Apple leaves upper epidermis layer peak height.

| Leaf# | Height (a. u.) | Leaf# | Height (a. u.) | Leaf# | Height (a. u.) | Leaf# | Height (a. u.) | Leaf# | Height (a. u.) | Leaf# | Height (a. u.) |
|-------|----------------|-------|----------------|-------|----------------|-------|----------------|-------|----------------|-------|----------------|
| 176   | 0.5030         | 183   | 0.6421         | 190   | 0.4503         | 197   | 0.501          | 204   | 0.5008         | 211   | 0.5226         |
| 177   | 0.5092         | 184   | 0.4254         | 191   | 0.4392         | 198   | 0.5476         | 205   | 0.4555         | 212   | 0.5536         |
| 178   | 0.4586         | 185   | 0.5066         | 192   | 0.4001         | 199   | 0.5195         | 206   | 0.4291         | 213   | 0.4953         |
| 179   | 0.4883         | 186   | 0.3818         | 193   | 0.5221         | 200   | 0.5053         | 207   | 0.5174         | 214   | 0.4528         |
| 180   | 0.5293         | 187   | 0.5232         | 194   | 0.5472         | 201   | 0.4932         | 208   | 0.5487         | 215   | 0.4057         |
| 181   | 0.5284         | 188   | 0.367          | 195   | 0.5222         | 202   | 0.4708         | 209   | 0.5239         | 216   | 0.4577         |
| 182   | 0.4057         | 189   | 0.4073         | 196   | 0.5113         | 203   | 0.6503         | 210   | 0.4889         | 217   | 0.3908         |

The disease name and the causative agents of the persimmon and apple leaves employed in this study are given in Table 1.

### Leaf inspection algorithm for persimmon and apple.

The algorithm for measuring the thickness between the PP and SP layers of persimmon using depth intensity profile is depicted in Fig. 4(a). The optical scanner, with the scanning position of the leaf, and the corresponding 2D OCT image of persimmon leaf with the upper epidermis (UE), PP, and SP layers are depicted in Fig. 4(a). A software program coded in Matlab (Mathworks, USA) was used to search for the intensity peak in the depth direction for the depth intensity profile analysis. After the 2D cross-sectional OCT image was loaded, RGB to grayscale conversion was performed. Then, a window was selected from the flattened 2D OCT image as a region of interest (ROI), marked with a red dotted box in Fig. 4(a), to apply a peak search algorithm during the depth intensity profile analysis. The peak search algorithm consecutively detected the highest intensity in each A-scan line. The unflattened 2D cross-sectional image contains the highest intensity index points at different positions in the lateral direction due to the physical structure of a leaf sample. Therefore, to get a flattened image, index positions with high intensity should be adjusted and matched linearly. Figure 4(b) shows the flattened image of the 2D OCT images shown in Fig. 4(a), with the thickness measuring ROI marked by the red dotted rectangle. A total of 160 A-scan lines were taken from the selected ROI, shown in the flattened image, and then summed up and averaged to get a single depth intensity profile for measuring the thickness of the persimmon leaf. The A-scan intensities were normalized by dividing them by their maximum values to obtain a stable intensity profile. Moreover, a median filter was used in the software program...
to compensate for the speckle noise to obtain a noise-free and smooth intensity plot. Figure 4(c) shows a single depth intensity profile of a selected ROI of a persimmon leaf. To obtain a more reliable depth intensity profile of a leaf, four ROIs were selected randomly from different positions on that leaf. Figure 4(d) shows the depth intensity profiles of four ROIs of a leaf were then summed and averaged to get a reliable depth intensity profile of a leaf in a single profile, as shown in Fig. 4(e). The average depth intensity profile of four ROIs was then curve fitted to obtain a smooth depth intensity profile of a single persimmon leaf, as shown in Fig. 4(f).

The algorithm used for evaluating apple leaf layer intensity peak width and height measurements is shown in Fig. 5. Image acquisition, grayscale conversion, flattening, filtering, depth profiling, and normalization of 2D cross-sectional OCT images (Fig. 5(a–c)) of apple leaves were performed using the same approach described for the persimmon leaf thickness measurement algorithm. The depth intensity profiles of three ROIs were then averaged to obtain a more reliable depth intensity profile for the apple leaf, as shown in Fig. 5(d). Finally, the width and height of the apple leaf layer intensity peaks were measured to detect healthy, apparently-healthy, and infected leaves. The width and height measurement process of apple leaf layer intensity peaks is shown in Fig. 5(e), where ΔW and ΔH indicate the width and height of the intensity peak, respectively. The custom code described in this section was developed according to a previously reported method58.

On-field qualitative inspection. OCT cross-sectional images of healthy, apparently-healthy, and infected persimmon and apple leaves are shown in Fig. 6. OCT cross-sectional images of healthy, apparently-healthy, and

Table 5. Apple leaves palisade parenchyma layer peak height.
Table 6. Apple leaves spongy parenchyma layer peak height.

infected persimmon leaves were visualized with UE, PP, and SP layers (marked with red arrows) and are shown in Fig. 6(a–c), respectively. The thickness difference between the UE and SP layers of healthy, apparently-healthy, and infected persimmon leaves, indicated using white arrows, is clearly distinguishable in OCT images. Similarly, Fig. 6(d–f) show the OCT cross-sectional images of healthy, apparently-healthy, and infected apple leaves, respectively. The thickness range of most healthy apple leaves is 60–80 

Quantified thickness-based thresholding. PP layer thickness differences between healthy, apparently-healthy, and infected specimens of persimmon leaf are shown in Fig. 7(a,b). In Fig. 7(a), the scatter plot presents the range of PP layer thickness of persimmon leaves, where it is visualized that the range of the PP layer thickness of healthy, apparently-healthy, and infected persimmon leaves is 100–160, 80–100, and >80 \( \mu \text{m} \), respectively. Figure 7(b) shows the average thickness of the PP layers of healthy, apparently-healthy, and infected persimmon leaves has an average thickness of 119.5, 89.8, and 72.1 \( \mu \text{m} \), respectively. The thickness range of most healthy apple leaves is 60–80 \( \mu \text{m} \), respectively. Figure 7(d) shows the average thickness of healthy, apparently-healthy, and infected apple leaves declined compared with that of healthy leaves. Figure 7(d) shows the average thicknesses
of healthy, apparently-healthy, and infected apple leaves to be 68.1, 59, and 31.7 µm, respectively. Moreover, the scatter plots in Fig. 7(a,c) present the ratio of healthy, apparently-healthy, and infected samples in persimmon and apple fields, respectively, where leaf samples were selected randomly.

Signal peak detection. Figure 8 shows the UE, PP, and SP layer intensity peak height/normalized intensity and width of healthy, apparently-healthy, and infected apple leaves. The method for measuring intensity peak height and width is discussed in Section 2.3. UE, PP, and SP layer intensity peak heights of apple leaves are shown in Fig. 8(a–c), respectively. As seen in Fig. 8(a), UE layer peak heights of healthy and apparently-healthy apple leaves are approximately the same, with the scatter plot showing no significant difference between them; however, the UE layer peak of infected leaves has declined compared with that of healthy and apparently-healthy leaves.

Table 7. Apple leaves upper epidermis layer peak width.

| Leaf# | Width (µm) | Leaf# | Width (µm) | Leaf# | Width (µm) | Leaf# | Width (µm) | Leaf# | Width (µm) | Leaf# | Width (µm) | Leaf# | Width (µm) |
|-------|------------|-------|------------|-------|------------|-------|------------|-------|------------|-------|------------|-------|------------|
| 1     | 27.5       | 23    | 25         | 45    | 32.5       | 67    | 25         | 89    | 30         | 111   | 32.5       | 133   | 35         |
| 2     | 25         | 24    | 27.5       | 46    | 30         | 68    | 35         | 90    | 32.5       | 112   | 32.5       | 134   | 35         |
| 3     | 25         | 25    | 27.5       | 47    | 27.5       | 69    | 25         | 91    | 30         | 113   | 32.5       | 135   | 35         |
| 4     | 27.5       | 26    | 27.5       | 48    | 30         | 70    | 32.5       | 92    | 27.5       | 114   | 32.5       | 136   | 35         |
| 5     | 27.5       | 27    | 27.5       | 49    | 25         | 71    | 30         | 93    | 32.5       | 115   | 32.5       | 137   | 35         |
| 6     | 25         | 28    | 22.5       | 50    | 32.5       | 72    | 32.5       | 94    | 30         | 116   | 32.5       | 138   | 35         |
| 7     | 27.5       | 29    | 30         | 51    | 25         | 73    | 32.5       | 95    | 35         | 117   | 30         | 139   | 35         |
| 8     | 27.5       | 30    | 32.5       | 52    | 30         | 74    | 32.5       | 96    | 30         | 118   | 32.5       | 140   | 35         |
| 9     | 25         | 31    | 30         | 53    | 27.5       | 75    | 32.5       | 97    | 30         | 119   | 32.5       | 141   | 35         |
| 10    | 27.5       | 32    | 27.5       | 54    | 27.5       | 76    | 30         | 98    | 30         | 120   | 30         | 142   | 35         |
| 11    | 25         | 33    | 27.5       | 55    | 27.5       | 77    | 30         | 99    | 32.5       | 121   | 30         | 143   | 35         |
| 12    | 30         | 34    | 27.5       | 56    | 27.5       | 78    | 32.5       | 100   | 32.5       | 122   | 30         | 144   | 35         |
| 13    | 22.5       | 35    | 30         | 57    | 27.5       | 79    | 30         | 101   | 30         | 123   | 37.5       | 145   | 37.5       |
| 14    | 22.5       | 36    | 25         | 58    | 25         | 80    | 32.5       | 102   | 30         | 124   | 32.5       | 146   | 30         |
| 15    | 25         | 37    | 30         | 59    | 27.5       | 81    | 22.5       | 103   | 30         | 125   | 35         | 147   | 30         |
| 16    | 27.5       | 38    | 27.5       | 60    | 27.5       | 82    | 27.5       | 104   | 30         | 126   | 30         | 148   | 35         |
| 17    | 27.5       | 39    | 27.5       | 61    | 27.5       | 83    | 32.5       | 105   | 35         | 127   | 30         | 149   | 35         |
| 18    | 27.5       | 40    | 27.5       | 62    | 30         | 84    | 32.5       | 106   | 32.5       | 128   | 30         | 150   | 35         |
| 19    | 27.5       | 41    | 32.5       | 63    | 30         | 85    | 32.5       | 107   | 32.5       | 129   | 30         | 151   | 32.5       |
| 20    | 27.5       | 42    | 30         | 64    | 27.5       | 86    | 32.5       | 108   | 32.5       | 130   | 30         | 152   | 35         |
| 21    | 25         | 43    | 30         | 65    | 27.5       | 87    | 30         | 109   | 30         | 131   | 35         | 153   | 35         |
| 22    | 25         | 44    | 30         | 66    | 30         | 88    | 30         | 110   | 32.5       | 132   | 32.5       | 154   | 37.5       |

| Leaf# | Width (µm) | Leaf# | Width (µm) | Leaf# | Width (µm) | Leaf# | Width (µm) | Leaf# | Width (µm) | Leaf# | Width (µm) | Leaf# | Width (µm) |
|-------|------------|-------|------------|-------|------------|-------|------------|-------|------------|-------|------------|-------|------------|
| 176   | 27.5       | 183   | 32.5       | 190   | 32.5       | 197   | 27.5       | 204   | 30         | 211   | 27.5       | 218   | 35         |
| 177   | 25         | 184   | 27.5       | 191   | 32.5       | 198   | 30         | 205   | 30         | 212   | 32.5       | 219   | 30         |
| 178   | 32.5       | 185   | 30         | 192   | 32.5       | 199   | 30         | 206   | 30         | 213   | 27.5       | 220   | 32.5       |
| 179   | 30         | 186   | 32.5       | 193   | 32.5       | 200   | 30         | 207   | 32.5       | 214   | 27.5       | 221   | 32.5       |
| 180   | 30         | 187   | 35         | 194   | 32.5       | 201   | 27.5       | 208   | 32.5       | 215   | 32.5       | 222   | 32.5       |
| 181   | 32.5       | 188   | 35         | 195   | 32.5       | 202   | 30         | 209   | 32.5       | 216   | 30         | 223   | 32.5       |
| 182   | 30         | 189   | 37.5       | 196   | 32.5       | 203   | 35         | 210   | 30         | 217   | 30         | 224   | 35         |

Table 7. Apple leaves upper epidermis layer peak width.
as shown in Fig. 8(e). Moreover, Fig. 8(e) reveals that the width of the PP layer intensity peak of healthy and apparently-healthy leaf specimens was nearly the same. The gradual decline of the intensity peak width of apparently-healthy and infected apple leaves compared with the healthy leaf specimens is plotted in Fig. 8(f). The SP layer peak merged with the PP layer peak in most apparently-healthy apple leaves. PP and SP layer peaks merged with the UE layer peak in infected apple leaves, and their plotting can be identified at the zero level of the scatter plot.

### Optical signal intensity assessments

A comparison of the average height and width of intensity peaks of healthy, apparently-healthy, and infected apple leaves is shown in Fig. 9. Figure 9(a) shows a comparison of the average height of intensity peaks. Intensities of the UE, PP, and SP layer peaks of apparently-healthy and infected apple leaves declined compared with healthy apple leaves. Notably, the PP and SP layer peaks disappeared in infected apple leaves due to merging with the UE layer peak. Figure 9(b) shows a comparison of the average width of intensity peaks, where not much difference between the UE layer intensity peaks of healthy, apparently-healthy, and infected apple leaves was observed. Moreover, the average width of the PP layer peaks of healthy and apparently-healthy is nearly the same. The average width of the SP layer peak of apparently-healthy leaves significantly declined compared with that of healthy leaves due to merging with the PP layer peak, as shown in Fig. 9(b). The PP and SP layer intensity peaks of infected apple leaves are not visible in Fig. 9(b) due to the merging of the PP and SP layer intensity peaks with the UE layer peak.

### Table 8. Apple leaves palisade parenchyma layer peak width.

| Leaf# | Width (µm) | Leaf# | Width (µm) | Leaf# | Width (µm) | Leaf# | Width (µm) | Leaf# | Width (µm) | Leaf# | Width (µm) | Leaf# | Width (µm) | Leaf# | Width (µm) | Leaf# | Width (µm) |
|-------|------------|-------|------------|-------|------------|-------|------------|-------|------------|-------|------------|-------|------------|-------|------------|-------|------------|
| Healthy (2nd Peak) | | | | | | | | | | | | | | | | | |
| 1 | 15 | 23 | 20 | 45 | 22.5 | 67 | 20 | 89 | 17.5 | 111 | 17.5 | 133 | 22.5 | 155 | 20 |
| 2 | 17.5 | 24 | 22.5 | 68 | 20 | 90 | 17.5 | 112 | 20 | 134 | 25 | 156 | 22.5 |
| 3 | 15 | 25 | 19 | 47 | 20 | 69 | 25 | 91 | 20 | 113 | 20 | 135 | 20 | 157 | 22.5 |
| 4 | 32.5 | 26 | 12.5 | 48 | 20 | 70 | 20 | 92 | 17.5 | 114 | 25 | 136 | 25 | 158 | 22.5 |
| 5 | 17.5 | 27 | 15 | 49 | 17.5 | 71 | 20 | 93 | 15 | 115 | 20 | 137 | 32.5 | 159 | 17.5 |
| 6 | 12.5 | 28 | 15 | 50 | 17.5 | 72 | 15 | 94 | 20 | 116 | 20 | 138 | 25 | 160 | 22.5 |
| 7 | 20 | 29 | 17.5 | 51 | 15 | 73 | 17.5 | 95 | 22.5 | 117 | 25 | 139 | 20 | 161 | 25 |
| 8 | 20 | 30 | 20 | 52 | 20 | 74 | 20 | 96 | 22.5 | 118 | 22.5 | 140 | 22.5 | 162 | 22.5 |
| 9 | 15 | 31 | 12.5 | 53 | 17.5 | 75 | 17.5 | 97 | 20 | 119 | 25 | 141 | 22.5 | 163 | 27.5 |
| 10 | 15 | 32 | 15 | 54 | 17.5 | 76 | 15 | 98 | 17.5 | 120 | 17.5 | 142 | 17.5 | 164 | 27.5 |
| 11 | 12.5 | 33 | 17.5 | 55 | 22.5 | 77 | 20 | 99 | 17.5 | 121 | 22.5 | 143 | 17.5 | 165 | 25 |
| 12 | 15 | 34 | 17.5 | 56 | 20 | 78 | 20 | 100 | 15 | 122 | 22.5 | 144 | 20 | 166 | 25 |
| 13 | 12.5 | 35 | 15 | 57 | 17.5 | 79 | 25 | 101 | 17.5 | 123 | 22.5 | 145 | 25 | 167 | 27.5 |
| 14 | 15 | 36 | 17.5 | 58 | 25 | 80 | 20 | 102 | 12.5 | 124 | 20 | 146 | 22.5 | 168 | 22.5 |
| 15 | 12.5 | 37 | 17.5 | 59 | 25 | 81 | 27.5 | 103 | 17.5 | 125 | 22.5 | 147 | 20 | 169 | 25 |
| 16 | 15 | 38 | 15 | 60 | 22.5 | 82 | 25 | 104 | 15 | 126 | 15 | 148 | 25 | 170 | 22.5 |
| 17 | 17.5 | 39 | 17.5 | 61 | 17.5 | 83 | 20 | 105 | 17.5 | 127 | 25 | 149 | 20 | 171 | 17.5 |
| 18 | 15 | 40 | 20 | 62 | 17.5 | 84 | 22.5 | 106 | 20 | 128 | 17.5 | 150 | 20 | 172 | 17.5 |
| 19 | 17.5 | 41 | 12.5 | 63 | 20 | 85 | 22.5 | 107 | 17.5 | 129 | 20 | 151 | 17.5 | 173 | 27.5 |
| 20 | 20 | 42 | 20 | 64 | 22.5 | 86 | 22.5 | 108 | 17.5 | 130 | 22.5 | 152 | 17.5 | 174 | 20 |
| 21 | 25 | 43 | 22.5 | 65 | 25 | 87 | 17.5 | 109 | 20 | 131 | 20 | 153 | 22.5 | 175 | 20 |
| 22 | 17.5 | 44 | 22.5 | 66 | 20 | 88 | 17.5 | 110 | 20 | 132 | 25 | 154 | 25 |

| Apparently-Healthy (2nd Peak) | | | | | | | | | | | | | | | | | |
| Healthy (2nd Peak) | | | | | | | | | | | | | | | | | |
| Infected (2nd Peak) | | | | | | | | | | | | | | | | | |

Table 8. Apple leaves palisade parenchyma layer peak width.
Our results revealed a decrease in PP layer thickness in apparently-healthy and infected persimmon leaves compared with healthy persimmon leaves. Moreover, the SP layer of apparently-healthy apple leaves merged with the PP layer. Similarly, the PP and SP layers merged with the UE layer in infected apple leaves; subsequently, a significant thickness difference was found in infected apple leaves. After detecting the significant thickness difference, a large-scale OCT image set was incorporated and averaged to set a reliable threshold for detecting healthy, apparently-healthy, and infected leaves. The average thicknesses of healthy, apparently-healthy, and infected apple leaves were 68.12, 58.95, and 31.72 µm, respectively; the average thicknesses of PP layers of healthy, apparently-healthy, and infected persimmon leaves were 119.46, 89.78, and 72.09 µm, respectively. In addition, the scatter plots (shown in Fig. 7) displayed the ratio and thickness variations of healthy, apparently-healthy, and infected leaves chosen randomly from persimmon and apple fields. The scatter plots (shown in Fig. 8) show variations in width and height of UE, PP, and SP layer intensity peaks of apple leaves.

The initial symptoms of plant diseases appear mostly on the leaf subsurface, which is a complex organ composed primarily of palisade parenchyma (PP) and spongy parenchyma (SP), crossed by vascular tissue, and enclosed by two epidermises. In this study, a developed depth profile algorithm was applied to cross-sectional OCT images of apple and persimmon leaves to quantitatively evaluate the inner structure of PP and SP layers, which are often seen in all types of leaves. As with all forms of leaf diseases, the PP and SP are affected, and their abnormalities can be identified by setting a threshold using the proposed method of this study, which allows this approach to work with different genotypes. Minimum Information About a Plant Phenotyping Experiment (MIAPPE) standard checklist has been given in the supplementary file.

### Table 9. Apple leaves spongy parenchyma layer peak width.

| Leaf# | Width (µm) | Leaf# | Width (µm) | Leaf# | Width (µm) | Leaf# | Width (µm) | Leaf# | Width (µm) | Leaf# | Width (µm) | Leaf# | Width (µm) | Leaf# | Width (µm) | Leaf# | Width (µm) | Leaf# | Width (µm) |
|-------|------------|-------|------------|-------|------------|-------|------------|-------|------------|-------|------------|-------|------------|-------|------------|-------|------------|-------|------------|
| 176   | 0          | 183   | 0          | 190   | 12.5       | 197   | 0          | 204   | 0          | 211   | 0          | 218   | 15         | 225   | 12.5       |
| 177   | 10         | 184   | 0          | 191   | 15         | 198   | 0          | 205   | 0          | 212   | 0          | 219   | 0          | 226   | 20         |
| 178   | 0          | 185   | 15         | 192   | 0          | 199   | 15         | 206   | 10         | 213   | 0          | 220   | 10         | 227   | 0          |
| 179   | 17.5       | 186   | 17.5       | 193   | 10         | 200   | 15         | 207   | 15         | 214   | 12.5       | 221   | 0          | 228   | 0          |
| 180   | 0          | 187   | 12.5       | 194   | 17.5       | 201   | 0          | 208   | 0          | 215   | 0          | 222   | 0          | 223   | 15         |
| 181   | 12.5       | 188   | 15         | 195   | 0          | 202   | 12.5       | 209   | 0          | 216   | 0          | 223   | 15         | 224   | 0          |
| 182   | 0          | 189   | 15         | 196   | 0          | 203   | 0          | 210   | 17.5       | 217   | 15         | 224   | 0          | 225   | 0          |
| 232   | 0          | 234   | 0          | 236   | 0          | 238   | 0          | 240   | 0          | 241   | 0          | 242   | 0          | 243   | 0          |
| 233   | 0          | 235   | 0          | 237   | 0          | 239   | 0          | 241   | 0          | 242   | 0          | 243   | 0          | 244   | 0          |

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Our results revealed a decrease in PP layer thickness in apparently-healthy and infected persimmon leaves compared with healthy persimmon leaves. Moreover, the SP layer of apparently-healthy apple leaves merged with the PP layer. Similarly, the PP and SP layers merged with the UE layer in infected apple leaves; subsequently, a significant thickness difference was found in infected apple leaves. After detecting the significant thickness difference, a large-scale OCT image set was incorporated and averaged to set a reliable threshold for detecting healthy, apparently-healthy, and infected leaves. The average thicknesses of healthy, apparently-healthy, and infected apple leaves were 68.12, 58.95, and 31.72 µm, respectively; the average thicknesses of PP layers of healthy, apparently-healthy, and infected persimmon leaves were 119.46, 89.78, and 72.09 µm, respectively. In addition, the scatter plots (shown in Fig. 7) displayed the ratio and thickness variations of healthy, apparently-healthy, and infected leaves chosen randomly from persimmon and apple fields. The scatter plots (shown in Fig. 8) show variations in width and height of UE, PP, and SP layer intensity peaks of apple leaves.

The initial symptoms of plant diseases appear mostly on the leaf subsurface, which is a complex organ composed primarily of palisade parenchyma (PP) and spongy parenchyma (SP), crossed by vascular tissue, and enclosed by two epidermises. In this study, a developed depth profile algorithm was applied to cross-sectional OCT images of apple and persimmon leaves to quantitatively evaluate the inner structure of PP and SP layers, which are often seen in all types of leaves. As with all forms of leaf diseases, the PP and SP are affected, and their abnormalities can be identified by setting a threshold using the proposed method of this study, which allows this approach to work with different genotypes. Minimum Information About a Plant Phenotyping Experiment (MIAPPE) standard checklist has been given in the supplementary file.
In this study, all OCT images of persimmon and apple leaves were acquired in-field and nondestructively using a backpack-type wearable OCT system from the persimmon and apple fields located in Daegu, South Korea. Obtained OCT images were employed to set a threshold for the pre-identification of palisade parenchyma (PP) and spongy parenchyma (SP) layer abnormalities of persimmon and apple leaves, which can subsequently reveal the symptoms of a diseased tree. However, three leaves were randomly chosen and imaged from each apple and persimmon tree. A total of 600 OCT images from 150 leaves (4 from each leaf), and 723 OCT images from 241 apple leaves (3 from each leaf) were used to set a threshold for the pre-identification of leaf abnormalities. Since, leaf layer abnormality is one of the major symptoms of plant disease, the proposed methodology of evaluating PP and SP layer abnormalities can be used to pre-identify the presence of disease in a tree. A zipped file, containing the cross-sectional OCT images (in.tif format) of apple and persimmon leaves has been uploaded to Figshare63. The measured persimmon and apple leaf layer thicknesses; widths and heights of normalized intensity peaks of UE, PP, and SP layers are listed in Tables 2 to 9.

**Technical Validation**

Our results can be technically validated using depth intensity profile analysis of persimmon and apple leaves. Depth intensity profiles of healthy, apparently-healthy, and infected persimmon leaves are shown in Fig. 10(a–c), Fig. 10(d–f), and Fig. 10(g–i), respectively. Black, blue, magenta, and green represent the depth intensity profiles of four ROIs of a single persimmon leaf. Figure 10(a,d,g) shows the depth intensity profiles of four ROIs of a single persimmon leaf. Figure 10(b,e,h) shows the average depth intensity profiles of four ROIs, and curve-fitted depth profiles of four ROIs of a single persimmon leaf, respectively. UE: upper epidermis, PP: palisade parenchyma, SP: spongy parenchyma.

Data Records

In this study, all OCT images of persimmon and apple leaves were acquired in-field and nondestructively using a backpack-type wearable OCT system from the persimmon and apple fields located in Daegu, South Korea. Obtained OCT images were employed to set a threshold for the pre-identification of palisade parenchyma (PP) and spongy parenchyma (SP) layer abnormalities of persimmon and apple leaves, which can subsequently reveal the symptoms of a diseased tree. However, three leaves were randomly chosen and imaged from each apple and persimmon tree. A total of 600 OCT images from 150 leaves (4 from each leaf), and 723 OCT images from 241 apple leaves (3 from each leaf) were used to set a threshold for the pre-identification of leaf abnormalities. Since, leaf layer abnormality is one of the major symptoms of plant disease, the proposed methodology of evaluating PP and SP leaf layer abnormalities can be used to pre-identify the presence of disease in a tree. A zipped file, containing the cross-sectional OCT images (in.tif format) of apple and persimmon leaves has been uploaded to Figshare63. The measured persimmon and apple leaf layer thicknesses; widths and heights of normalized intensity peaks of UE, PP, and SP layers are listed in Tables 2 to 9.
Fig. 11 Depth profiles of healthy, apparently-healthy, and infected apple leaves. (a, and b), (c, and d), and (e, and f) show depth profiles of healthy, apparently-healthy, and infected apple leaves, respectively. (a, c, and e) depth intensity profiles of three regions of interest (ROIs) from single healthy, apparently-healthy, and infected apple leaves, respectively. (b, d, and f) averaged depth intensity profiles of three ROIs of a single leaf of healthy, apparently-healthy, and infected apple leaves, respectively. UE: upper epidermis, PP: palisade parenchyma, SP: spongy parenchyma.

depth intensity profile of four ROIs of a single leaf, values of PP layer thicknesses of healthy, apparently-healthy, and infected persimmon leaves were 118, 92, and 73 µm, respectively. PP layer thickness gradually decreased from healthy to apparently-healthy and infected persimmon leaves, where the UE and SP layers were the same for all.

Depth intensity profiles of healthy, apparently-healthy, and infected apple leaves are shown in Fig. 11(a–f), respectively. Black, blue, and magenta represent depth intensity profiles of three ROIs from a single apple leaf. Figure 11(a,c,e) show depth intensity profiles of three ROIs from healthy, apparently-healthy, and infected apple leaves, respectively. The algorithm for obtaining the depth intensity profile of a single leaf is described in Section 2.3. Figure 11(b,d,f) shows average depth intensity profiles of three ROIs from single healthy, apparently-healthy, and infected apple leaves, respectively. In the average depth intensity profile of a healthy leaf, shown in Fig. 11(b), three distinguishable intensity peaks can be visualized, which show the presence of three distinct layers in a healthy leaf, indicated by black arrows. In the average depth profile of an apparently-healthy leaf, shown in Fig. 11(d), the intensity peaks of the PP and SP layers merged (blue arrows) to appear as a single peak, which is separate from the UE layer peak. Finally, in the average depth profile of an infected apple leaf, shown in Fig. 11(f), the intensity peaks of the UE, PP, and SP layers merge (red arrow) and appear as a single peak.

Code availability
Two separate MATLAB programs were used for flattening the captured OCT images and for A-scan profiling. MATLAB version 8.3 (R2014a) was installed for running the script and there was no special requirement for this analysis. First, the OCT image needs to be loaded in the image flattening program, and then the mouse pointer needs to be dragged from the right to the left of the image to get the desired flattened image. The ROI of the
flattened section can be adjusted. After saving the flattened OCT images, the A-scan profiling program needs to be applied to the saved flattened images to get their A-scan profiles. The details documentation of the MATLAB script will help to reuse the code. From A-scan profiles, the width and height of UE, PP, SP layer intensity, and leaf thickness can be measured to estimate and set the threshold for pre-identification of leaf abnormalities. The leaf layer intensity and thickness measurement process has already been discussed in the 'leaf inspection algorithm for persimmon and apple' section in the methods part. The MATLAB program files named 'Image_flattening.m' and 'A_scan_profiling.m' have been uploaded to Figshare.

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Author contributions
Mansik Jeon, Hee-Young Jung, and Jeehyun Kim jointly conceived the initial concept. Sm Abu Saleah and Ruchire Eranga Wijeingshe performed the main research and analysis tasks of this work and wrote the manuscript. Sm Abu Saleah, Daewoon Seong, and Seung-Yeol Lee conducted data analysis and developed the method. Ruchire Eranga Wijeingshe and Naresh Kumar Ravichandran provided the code for the experiments and helped in data collection and general method development. Mansik Jeon and Hee-Young Jung guided in developing the method and reviewed the manuscript. Mansik Jeon and Jeehyun Kim supervised the work and arranged funds for this work. All authors reviewed and approved the manuscript.

Competing interests
The authors declare no competing interests.
Additional information

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