Functional optical coherence tomography (fOCT) biospeckle imaging to investigate response of plant leaves to ultra-short term exposure of Ozone

L K T Srimal¹,2, U M Rajagopalan³ and H Kadono¹

¹Graduate School of Science and Engineering, Saitama University, 255 Shimo-okubo, Sakura-ku, Saitama-shi, Saitama-ken 338-8570, Japan,
²Department of Mechanical and Manufacturing Engineering, Faculty of Engineering, University of Ruhuna, Hapugala, Galle 80000, Sri Lanka
³Faculty of Food Life Sciences, Toyo University, 1-1-1 Izumino, Itakura-machi, Oragun, Gunma-ken 374-0113, Japan

E-mail: thanujasrimal@gmail.com

Abstract. In this study, response of leaves of Chinese chives (Allium tuberosum) to ozone stress was investigated using functional optical coherence tomography (fOCT) based on biospeckle. The biospeckles arising out of dynamic motion of organelles can reflect the biological activities of plant. The fOCT biospeckle image was obtained by calculating the standard deviation (SD) of the fOCT temporal signal (biospeckle signal) at each and every point from the successively acquired OCT images. Plant leaves were subjected to treatment under different concentrations of O₃, and imaging data were acquired from back and front surfaces of the leaves. The internal cell structure within the Chinese chives leaves could be clearly visualized in the functional OCT biospeckle image, which was not clearly visible in conventional OCT cross-sectional image. The SDs were found to be increasing significantly, especially in the surface layers of both front and back sides of the leaf with ozone exposure. Thus, the fOCT based on biospeckle is found to be suitable for fast, non-destructive monitoring environmental stresses on plants, which can potentially lead to significant time saving, for which conventional techniques require a few days to a few weeks time.

1. Introduction

Ozone (O₃) is generally conceived as occurring only in the form of beneficial stratospheric O₃, a natural screen from the harmful effects of ultraviolet (UV) radiation. Although stratospheric O₃ is decreasing, concentration of surface O₃ in the troposphere (tropospheric O₃) is increasing due to complex photochemical reactions involving volatile organic compounds (VOCs) and nitrogen oxides (NOx) in the presence of UV light [1]. For example, tropospheric O₃ concentrations exceeding 80ppb occur regularly, in China, Japan, and even in rural areas of India [2].

Tropospheric O₃ enters the leaf through stomata, the pores for photosynthetic gas exchange. It is known that O₃ causes biochemical and physiological changes leading to the direct or indirect inhibition of photosynthesis, extra cost of repairing any cellular and metabolic damage, and a consequent decrease in plant growth. Adverse effects on plant photosynthesis were identified as a
major factor limiting growth and yields of crop under high O$_3$ concentrations [3]. Yield loss is caused by the cumulative effect of chronic daily O$_3$ exposure over the growth period [4].

Biomass, height change, and counting are commonly employed techniques to monitor the effect of O$_3$ stress on plants [5]. Those require long waiting periods, and the changes in the internal structure of the plant cannot be recognized in real time. So, there is a need for fast and reliable methods to assess the structural (i.e., functional) changes in plants against O$_3$ stress. The majority of methods for investigating internal structure of plants are invasive. They cause damage to the tissue under study, thereby limiting their application for in-vivo monitoring. An example of such a destructive approach is conventional optical microscopy which requires a destruction of the sample and a subsequent analysis, thus plants responses to external stress are inferred indirectly. In addition, the analysis cannot be performed in real time.

To overcome these limitations, we proposed to use optical coherence tomography (OCT). OCT [6] is an interferometric technique that detects internal reflected light and can provide non-contact two or three dimensional in-vivo tomographic images of the internal tissue structure with very high resolution of a few micrometers. It has generated numerous biological applications, most commonly in the fields of ophthalmology [7] and dermatology [8]. More recently, OCT has also been used in the field of botany to visualize the inner structure of tissue [9] and to study plants suffering from pathogen attack with conventional structural OCT imaging [10]. However, its potential in monitoring functional changes, in other words, biological activities inside tissue has not been well developed. In this study, we proposed to use biospeckle signal to realize a functional OCT (fOCT) [11] to monitor the effect of O$_3$ on plants.

Processes such as cytoplasmic streaming, organelle movement, cell growth and division, and biochemical reactions are the biological activities occurring inside of plant leaf [12]. Behavior of these activities are changed under the influence of environmental stresses. We developed, and applied, proposed fOCT based on biospeckle to monitor the changes in biological activities of plants. Biospeckle is optical information that has been getting attention during last fifteen years for evaluation of properties of biological materials to monitor biological activities of plants [13].

The speckle pattern is generated by interference created by the backscattering of a random distribution of scatters at some distance from a scattering object under the illumination of a coherent light such as a laser. If there is no movement in scatters and no change in the structure of the object, the speckle pattern is stable in time. However, in the case of biological samples, the speckle pattern shows a dynamic property in time due to the moving organelles and particles within the sample. This dynamic speckle pattern is characteristic for biological tissues and has been called a biospeckle [14]. Therefore, the dynamic characteristics of the biospeckle can reflect the biological activities of a living substance [15].

To realize the biospeckles fOCT, OCT cross-sectional images were acquired successively. Magnitude of the biospeckle signal, in other words, standard deviation (SD) of the signal was calculated along time axis of each spatial position from the array of OCT cross-sectional images. Then a fOCT biospeckle image was constructed from the SDs of biospeckle signals. Magnitude (SD) of the time varying biospeckle signal reveal different biological information and it was proposed as a measure to evaluate the changes in biological activities against O$_3$ stress on plants.

In the experiments, Allium tuberosum, commonly known as Chinese chives was exposed to different concentrations of O$_3$, and the effects to both back and front side of the leaf were observed to validate the developed fOCT based on biospeckle.

2. Optical System and Method

2.1. Optical system

SD-OCT system was constructed in this study to achieve high resolution OCT imaging. The schematic diagram of the SD-OCT system is shown in Figure 1. The light source used in the system is a super luminescent diode (SLD) with a central wavelength 836.1nm and a band width of 55.2nm. A 1x2
fiber coupler splits the incident light into sample (99%) and the reference (1%) beams. Reference arm consist of collimating lens L1, objective lens L4, and mirror M1. The backscattered light from the sample and the light reflected from the reference mirror are recombined by the coupler. By means of a circulator, those lights are guided into a spectrometer consisting of a collimator, lens L5, and grating. The collimated light enters grating, and the spectral interference signal is obtained by the line-scan camera through lens L6. We used a line scan CCD camera (L104k-2k, BASLER, Germany) with 2048 pixels and acquisition rate of 25,000 lines per second. The axial resolution (depth resolution) is given by [16],

$$\Delta z = \frac{2 \ln \lambda_o^2}{\pi n \Delta \lambda},$$

where, \(\lambda_o\), \(\Delta\) are central wavelength, and bandwidth of the light source, respectively, \(n\) is the refractive index of the tissue. The depth resolution in free space was estimated to be 6\(\mu\)m in and the lateral resolution was estimated to be 22\(\mu\)m (NA=0.024).

$$\Delta z = \frac{2 \ln \lambda_o^2}{\pi n \Delta \lambda},$$

Figure 1. Experimental setup of spectral domain optical coherence tomography system. SLD: super luminescent diode, L1-L6: lenses, M1: mirror, PC: polarization controller, DAQ: data acquisition device, IMAQ: image grabber.

Galvano-mirrorscanners in the sample arm permits a lateral scanning in x and y directions. The incident power delivered to the leaf sample was approximately 5mW, that gave an irradiance lower than the damage threshold for plant leaves [17].

2.2. Standard deviation of biospeckle signal

Acquired spectral interference signal from the line scan camera was first transformed into wave number, \(k\), space from wavelength, \(\lambda\), space that is called as a rescaling. Next, the signal was Fourier transformed in order to obtain the depth resolved reflectivity profile of the sample at the focal spot of the sample beam for a fixed lateral position (A-scanning). multiple A-scans were performed while the galvano-mirror swept the probing beam across the sample, and the sequentially acquired line image (B-scanning) were stored in the computer into a two-dimensional cross-sectional image of the sample. One image of size 1024x2500 pixels was acquired with an acquisition rate of 10 frames per second.

A certain target region of the leaf image of size 65x2500 pixels (223x4000 \(\mu\)m\(^2\)) acquired at time \(t_i\) was chosen from the whole cross-sectional image \(I_{OCT}(x,y;t_i)\). Then, a fOCT biospeckle image \(I_b(x,y;t_i)\) was obtained by calculating the standard deviation (SD) of the fOCT biospeckle signal at each and every point in the selected portion of the OCT cross-sectional image along the time axis (from the successively acquired OCT cross-sectional images) as given by,
\[ I_b(x, y, t) = \left[ \frac{1}{N} \sum_{j=1}^{N} \left( I_{OCT}(x, y, t + j) - \langle I_{OCT}(x, y, t) \rangle \right)^2 \right] \frac{1}{2} \] (2)

Where,

\[ \langle I_{OCT}(x, y, t) \rangle = \frac{1}{N} \sum_{j=1}^{N} I_{OCT}(x, y, t + j) \] (3)

and \( N \) is the number of averaging frames.

2.3. Plant and ozone exposure system

The \( \text{O}_3 \) exposure system consists of air pump, charcoal filters (CF), an \( \text{O}_3 \) generator (OES 10A, Dylec Inc., Japan), an \( \text{O}_3 \) monitor (Model 1150, Dylec Inc., Japan), main \( \text{O}_3 \) chamber (33.5×33.5×58 cm\(^3\)). A specially designed leaf \( \text{O}_3 \) chamber (10×10×2 cm\(^3\)), made up of plastics, was connected to the main \( \text{O}_3 \) chamber to the leaf of the plant under \( \text{O}_3 \) exposure. Generated \( \text{O}_3 \) was fed into the main \( \text{O}_3 \) chamber, and its concentration was precisely controlled by measuring the \( \text{O}_3 \) concentrations with the \( \text{O}_3 \) monitor and feeding the signal back to the generator. The \( \text{O}_3 \) generation system approximately took 9 minutes, 10 minutes, and 14 minutes to reach the target \( \text{O}_3 \) concentrations of 80 ppb, 120 ppb, and 240 ppb, respectively. The \( \text{O}_3 \) feedback control system could precisely stabilize the concentration of \( \text{O}_3 \) within the leaf and main \( \text{O}_3 \) chambers at an accuracy of around 0.5% during the experiments.

![Figure 2. Schematic of \( \text{O}_3 \) exposure system used in this study.](image)

**Figure 2.** Schematic of \( \text{O}_3 \) exposure system used in this study.

CF: charcoal filter, FB: feed back signal.

Chinese chives plants were grown in a growth chamber (Conviron, Controlled Environmental Ltd, Canada) for 30 days. We maintained the relative humidity at 55%-65%, and respective day/night cycle of 12/12 hours at a light irradiance of 260-350 \( \mu \text{molm}^{-2}\text{s}^{-1} / 0 \ \mu \text{molm}^{-2}\text{s}^{-1} \) and an air temperature of 25°C/18 °C. The plants were kept regularly watered. In the experiment, healthy young leaves of the plants, one to two weeks old, were used and mounted in the leaf \( \text{O}_3 \) chamber. Three halogen lamps with the fiber optic light guides (PHL-150, MEJIRO PRECISION, Japan) were used to illuminate the plant leaf with a light irradiance of 500 \( \mu \text{molm}^{-2}\text{s}^{-1} \) during experiments.

2.4. Timing protocol and data acquisition

The experiments were carried out with Chinese chives under \( \text{O}_3 \) concentrations of 0, 80, 120, and 240 ppb to investigate the influence of \( \text{O}_3 \). The entire experimental duration was 8.5 hours (Figure 3). As an initial condition, a continuous air flow (10 liter/min.) after passing the CF was fed into the leaf \( \text{O}_3 \) chamber (Figure 2), where the leaf was mounted, for two hours to acclimate to the environmental condition prior to the experiment. After acclimation, for each \( \text{O}_3 \) exposure condition, next half an hour
was allowed for settling of the desired O₃ concentration levels (0, 80, 120, and 240 ppb) in the main O₃ chamber. At the end of this half an hour period, the air containing O₃ with the desired concentration will reach leaf without passing through the CF by switching off the valve as shown in Figure 2. The O₃ exposure was carried out for three hours followed by another three hours of CF air. The O₃ concentrations of 120 ppb and 240 ppb were chosen based on the criterion used in Japan, where a warning and a serious warning are issued for 120 ppb and 240 ppb of O₃ concentrations, respectively, in Japan. Meantime, 0 ppb was chosen for control experiments. Both the temperature and the humidity were kept constant during a single session of experiment with respective variations, ±2% and ±1% respectively. Measurements were performed at the position 5 cm away from the apex of the leaf.

![Figure 3. Timing protocol of O₃ exposure and data acquisition.](image)

OCT imaging data were acquired (Figure 3) sequentially, before, and at 1 hour, 2 hours, and 3 hours after starting O₃ exposure (within), and at 1 hour, 2 hours, and 3 hours after stopping of O₃ exposure (after). Same procedure was applied to the measurements for the front and back surfaces of leaves identically. O₃ concentrations of 0, 80, 120, and 240 ppb were used. Six replicate experiments were performed under each O₃ concentration.

3. Results and discussion

3.1. Anatomy

Micro-structural cross-sectional observation was done for a Chinese chives leaf (Figure 4). Epidermal cells (10 µm thick layer), oval shape mesophyll cells (around 100 µm in height), vascular bundle, stomata were clearly visible. Here, epidermal layer is the outer most cell layer of the plant leaf, which protects leaf against water loss, and regulate gas exchange. It is a single layered structure. Mesophyll cell is the primary location of photosynthesis in the leaf. And these cells govern most of the leaf interior. Vascular tissues help for the transport system for fluids and nutrient internally. They are arranged in long, discrete strands called vascular bundle [18].

![Figure 4. Optical microscopy analysis of cross-section of a leaf of Chinese chives. uc:upper epidermis, mc:mesophyll cell, st:stomata, vb:vascular bundle.](image)

3.2. OCT observation-structural and biospeckle imaging

Conventional OCT cross-sectional imaging can visualize internal structure of biological tissues. Figure 5(a) shows conventional OCT cross-sectional image of a Chinese chives leaf. Epidermal cell layer could be clearly seen. However, layer such as mesophyll cells was not clearly visible. Figure 5(c) shows corresponding fOCT biospeckle image. The depth structure became clear. Comparing with the
microscopic image in Figure 4, upper epidermal layer and the distinctive more oval shaped mesophyll cells were clearly seen in the fOCT biospeckle image.

Figure 5. Conventional structural OCT cross-sectional images (a) before exposure of O₃, (b) 3 hours after starting O₃ exposure. Corresponding fOCT biospeckle images (c) before exposure of O₃, (d) 3 hours after starting O₃ exposure. Magnified views of the mesophyll cell layer encased in black dotted squares shown in (a), (b), (c), and (d) are shown, respectively in (e), (f), (g), and (h). ue: upper epidemis, mc: mesophyll cell, ve: vesicular bundle, st: stomata.

3.3. OCT observation - O₃ effect on leaf

Figure 6. (a) Depth profiles of selected area of conventional structural OCT cross-sectional images, (b) Corresponding depth profiles of fOCT biospeckle images.
The experiments of O3 exposure were conducted according to the timing protocol described in section 2.4. Figures 5(a), and (b) show conventional structural OCT image of Chinese chives leaf (back surface) acquired before O3 exposure, and 3 hours after starting O3 exposure, respectively. Figures 5(c), and (d) represent the corresponding fOCT biospeckle (SD) images before O3 exposure, and 3 hours after starting O3 exposure. It was difficult to find a clear difference in conventional OCT images with O3 exposure (Figures 5 (a), (b), (e), and (f)). However, a variation due to O3 exposure could be seen in the fOCT biospeckle images (Figures 5(c), (d), (g), and (h)). Oval shaped periodic structure that corresponds to mesophyll cells became unclear with even 3 hours short term O3 exposure in biospeckle images. Earlier studies confirmed that of cells belonging to mesophyll layer as the main target of O3 injury [19]. So, biospeckle signals coming from different depth layers were analyzed for their temporal characteristics (Unpublished).

Laterally averaged depth profile of conventional structural OCT images so as to improve S/N ratio were obtained under conditions of before, 1, and 3 hours after starting as well as 2 hours after stopping O3 exposure and are shown in Figure 6(a). Figure 6(b) shows corresponding depth profiles of the fOCT biospeckle images. Here, laterally averaged depth profiles, were obtained from the lateral position of 0 to 1000 µm as indicated by the dark dotted line in the OCT structural image and the corresponding biospeckle image (Figures 5(a), and (c)).

Under all the different O3 exposure conditions, maximum values were obtained at a depth position of 25.3 µm in the conventional structural OCT images, and it corresponded to the upper epidermal layer of the leaf. The variation of the values between before and after 3 hours of O3 exposure was 3.8%. On the other hand, the corresponding value of fOCT biospeckle images was significantly increased to 44%. This variation with O3 exposure in the depth profile of fOCT biospeckle image was larger for epidermal layer in comparison the other deeper layers. Here, the effect of O3 on mesophyll cell layer seen in Figures 5(g) and (h) was degraded in the depth profile of fOCT biospeckle images due to the lateral average. Therefore, the maxima of averaged depth profiles corresponding to the epidermal layer of fOCT biospeckle images at each time interval such as before, after starting and after stopping of O3 exposure were used for further analysis in following discussion.

In order to evaluate the effect of exposure of O3, a normalized parameter was introduced. SDs of biospeckle signals obtained from the epidermal layer corresponding to a depth position of 25.3 µm under O3 exposure conditions were normalized by the SDs obtained before O3 exposure, SDb. Furthermore, averaged normalized standard deviation (ANSD) was defined as,

\[
\text{ANSD} = \frac{\langle SD \rangle_{\text{within}}}{\langle SD \rangle_{b}},
\]

where, \(\langle \ldots \rangle\) denotes average over 18 measurements. The normalized SDs were calculated across each hour of 1 hour, 2 hour and 3 hours and averaged over six different samples thus totaling to 18 measurements. The term ‘within’ was used to refer to the average of SDs after starting O3 exposure while term ‘after’ referred to the average after stopping O3.

The measurements for back and front sides of the leaf were performed independently as a different series of experiments. The experimental results for the back and front surfaces of the leaf are shown in Figures 7(a), and (b), respectively for O3 concentrations of 0, 80, 120, and 240 ppb.

It can be seen from the Figures 7(a), and (b) that SD becomes larger for both back and front surfaces as the concentration of O3 becomes larger. For the back surface of the leaf, the differences in ANSDs between before and within O3 exposure to 80, 120, and 240ppb were significant (t-test, \(P < 0.01\), Figure 7(a)). In addition, the differences in ANSDs between before and after O3 exposure for 80, 120, and 240ppb were also significant (t-test, \(P < 0.01\), Figure 7(a)).

Comparing Figures 7(a), and (b), the ANSDs of the front surface were smaller than those on back surface. For the front surface of the leaf, the differences in ANSDs between before and within, and
also before and after O$_3$ exposure of 240 ppb concentration were significant (t-test, $P < 0.01$, Figure 7(b)). However, the differences in ANSDs between before and within, and also before and after O$_3$ exposure to 80, and 120 ppb concentrations were not significant.

Figure 7. ANSDs of biospeckle signals of Chinese chive leaves under O$_3$ exposures of 0, 80, 120, and 240ppb. Each average value is plotted with standard error for 18 measurements, for (a) back surface, and (b) front surface. The ANSD within O$_3$ exposure and after exposure were compared with that before exposure using statistical two samples t-test. (a, $P < 0.01$; b, not significant).

The differences in ANSD between the front and back surfaces for concentrations of 120 ppb and 80 ppb O$_3$ exposure were 26.7% and 13%, respectively. On other hand, the corresponding values for 240 ppb O$_3$ exposure was reduced to 13%. This may due to the saturation of the reaction to O$_3$ exposure of the back surface of the leaf. For the different responses of the front and back sides of the leaf for O$_3$ exposures, the stomata may play a significant role since O$_3$ is taken up through the stomata.

3.4. Stomatal influence on ANSD under O$_3$ exposure

O$_3$ taken up through stomata reacts with water and intercellular components to generate highly reactive oxygen species (ROS), that include peroxides and free radicals. The free radicals are probably believed to be the real cause of the negative effect of O$_3$. Plants have a number of mechanisms that chemically and enzymatically remove these toxic compounds avoiding cellular damage [20]. It may be the reason for increase of fluctuation of biospeckle signal coming from outermost epidermal layer. O$_3$ stress affects the photosynthetic activity while altering leaf and root respiration rates [21]. Therefore, stomata densities on both sides of the leaves were examined. Figure 8 shows a microstructural observation of epidermal layer removed from a Chinese chives leaf. Number of stomata was counted within a circular area of radius 940 µm, and the stomata densities were calculated. The stomata densities on the front and back sides of the leaves were 11.1±1.7 and 12.3±1.5, respectively. Therefore, a significant difference could not be found.
Moreover, from the cross-sectional image of the leaf shown in Figure 4, it was difficult to find a clear structural difference between front and back sides. However, roughness of back surface of the leaf is larger and hence has a larger surface area than front surface. This may result in the difference in the total number of stomata on the front and back surfaces. In addition, stomatal conductances may be different for the front and back surfaces. However, they remain the focus for our future research.

4. Summary
We proposed, developed, and applied the fOCT biospeckle imaging technique for monitoring O₃ stress on plants. The internal cell structure, specially mesophyll cell layer, within the Chinese chives leaf can clearly been seen in the fOCT biospeckle image, which is not clearly visible in conventional OCT cross-sectional image. Furthermore, it was experimentally demonstrated that the influence of O₃ on each plant structure, e.g., epidermal and mesophyll cell layers, could be individually monitored even for short-term O₃ exposures.

In this study, we found that, epidermal layers of both surfaces of the leaf showed an increased fluctuation in biospeckle signal under the O₃ stress. Further, the study also found a larger fluctuation against O₃ exposure for back side than those for front surface. These results indicate that the epidermal layer of back side of Chinese chives leaf is more sensitive to O₃ stress than the front surface.

The presented study suggests possible applications of developed fOCT biospeckle imaging and signal analysis technique, for investigating the immediate impact of environmental factors. Especially even for O₃ exposure, for a very short span of time such as a number of seconds or a few minutes the proposed fOCT biospeckle was found to be effective which is not possible with conventional experimental methods and even with conventional OCT imaging.

Acknowledgements
This work was partly supported by the Grant-in-Aid for Science Research in JSPS (No. 24651023) of the Ministry of Education, Culture, Sports, Science and Technology in Japan.

References
[1] Oltmans S J et al 1998 Geophysical Research Letters 25 139–42
Ashmore M R 2005 Plant, Cell and Environment 28 949–64
Rathnayake A P, Kadono H, Toyooka S and Miwa M 2007 Journal of Forest Research 12 393–402
Ainsworth E A 2008 Global Change Biology 14 1642–50
[2] Thilakarathne B L S, Rajagopalan U M, Kadono H and Yonekura T 2014 SpringerPlus 3 89
[3] Kobayashi K and Okada M 1995 Agriculture, Ecosystems and Environment 53 1–12
[4] Lefohn A S 1992 Surface-level Ozone Exposure and Their Effects on Vegetation (Lewis Publishers) pp157–270
[5] Díaz-de-Quijano M, Schaub M, Bassin S, Volk M and Peñuelas J 2012 Environmental Pollution 169 250–7
[6] Huang D et al 1991 Science 254 1178–81
Fercher A F 1996 Journal of Biomedical Optics 1 157–73
[7] Drexler W, Morgner U, Ghanta R K, Kärtner F X, Schuman J S and Fujimoto J G 2001 *Nature Medicine* **7** 502–7
    Wojtkowski M, Leitgeb R, Kowalczyk A, Bajraszewski T and Fercher A F 2002 *Journal of Biomedical Optics* **7** 457–63

[8] Gambichler T, Moussa G, Sand M, Sand D, Altmeyer P and Hoffmann K 2005 *Journal of Dermatological Science* **40** 85–94

[9] Hettinger J W, Mattozzi M D L P, Myers W R, Williams M E, Reeves A, Parsons R L, Haskell R C, Petersen D C, Wang R and Medford J I 2000 *Plant Physiology* **123** 3–15
    Reeves A, Parsons R L, Hettinger J W and Medford J I 2002 *Journal of Microscopy* **208** 177–89

[10] Boccara M, Schwartz W, Guiot E, Vidal G, Paepe R D, Dubois A and Boccara A C 2007 *The Plant Journal* **50** 338–46

[11] Maheswari R U, Takaoka H, Homma R, Kadono H and Tanifuji M 2002 *Optics Communications* **202**, 47–54
    Maheswari R U, Takaoka H, Kadono H, Homma R and Tanifuji M 2003 *Journal of Neuroscience Methods* **124** 83–92
    Chen Y, Aguirre A D, Ruvinskaya L, Devor A, Boas D A and Fujimoto J G 2009 *Journal Neuroscience Methods* **178** 162–73

[12] Braga R A, Dupuy L, Pasqual M and Cardoso R R 2009 *European Biophysics Journal* **38** 679–86

[13] Kurenda A, Adamiak A and Zdunek A 2012 *Postharvest Biology and Technology* **67** 118–23

[14] Aizu Y and Asakura T 1996 *Trends in Optics* (San Diego: Academic Press) pp27–49

[15] Ansari M D Z and Nirala A K 2013 *Optik* **124** 2180–86

[16] Fujimoto J G and Drexler W 2008 *Optical Coherence Tomography: Technology and Applications* (Springer)

[17] Chow T H, Tan K M, Ng B K, Razul S G and Tay C M, 2009 *Journal of Biomedical Optics* **14** 014006

[18] Taiz L and Zeiger E 2002 *Plant Physiology* (Sunderland: Sinauer Associates)

[19] Vollenweider P, Ottiger M and Goerg M S G 2003 *Environmental Pollution* **124** 101–18
    Faoro F and Iriti M 2009 *Environmental Pollution* **157** 1470–77

[20] Kangasjärvi J, Talvinen J, Utriainen M and Karjalainen R 1994 *Plant, Cell and Environment* **17** 783–94
    Sharma Y K and Davis K R 1997 *Free Radical Biology & Medicine* **23** 480–88
    Sandermann H, Ernst D, Heller W and Langebartels C 1998 *Trends in Plant Science* **3** 47–50
    Dizengremel P 2001 *Plant Physiology and Biochemistry* **39** 729–42
    Calatayud A, Iglesias D J, Talón M and Barreno E 2003 *Plant Physiology and Biochemistry* **41** 839–45

[21] Walter A and Schurr U 2005 *Annals of Botany* **95** 891–900

[22] Kinose Y, Azuchi F, Uehara Y, Kanomata T, Kobayashi A, Yamaguchi M and Izuta T 2014 *Environmental Pollution* **194** 235–45