Creating Seed Coat Catalog Using Spectral Domain Optical Coherence Tomography

Xinhua Li
Dept. of Electrical Engineering and Electronics
University of Liverpool
Liverpool, UK
xinhua.li@liverpool.ac.uk

Xingyu Yang
Dept. of Electrical Engineering and Electronics
University of Liverpool
Liverpool, UK
sgxyang5@student.liverpool.ac.uk

Zijian Zhang
Dept. of Electrical Engineering and Electronics
University of Liverpool
Liverpool, UK
Z.Zhang116@liverpool.ac.uk

Dingming Kang
Dept. of Agronomy and Biotechnology
China Agricultural University
Beijing, China
kdm@cau.edu.cn

Yaochun Shen
Dept. of Electrical Engineering and Electronics
University of Liverpool
Liverpool, UK
ycshen@liverpool.ac.uk

***Abstract*— As a non-destructive method, spectral-domain optical coherence tomography (OCT) has already been adapted to study seed samples. SD-OCT provides a set of capabilities in terms of millimetre-scale penetration depth, high-speed acquisition, and resolution typically in the range of ten microns. It has been therefore gaining popularity in this specific field. However, only a limited number of seed cultivars have been investigated with OCT so far, and , and there has been little discussions about the applicability of the OCT to other seed varieties. In this work, we report the measurement of 14 types of seed using OCT system. It was found that OCT can provide high-resolution cross-sectional images of 12 out of 14 seed types. This work represents the first systematic study towards a seed catalog which provides the essential information about the applicability of OCT for studying a variety of seeds.**

Keywords—optical coherence tomography, seed catalog, seed coat thickness

I. INTRODUCTION

Optical coherence tomography (OCT) is a non-destructive imaging method that has recently attracted interests in the study of seed.. It offers fast imaging speed, high resolution and the ability to resolve the internal structures [1]. OCT has already been applied for seed hull thickness measurement [2], seed disease detection by observing the abnormal layer thickness of the seed [3, 4], and rice seed classification [5]. In addition, OCT has also been used to monitor the capsicum annuum seed growth under different solution concentrations. Of particular interest, the seed coat is an essential structure of seeds. The permeability of seed coats can restrict the exchange of water, gas, and nutrients between the embryo and the outside, affecting the growth and metabolism of seeds [6].The seed coat is also a crucial factor affecting the life expectancy of seeds as it protects the embryo. The looseness of the seed coat structure is closely related to the metabolism of seeds. The palisade cells in the seed coat contain a large number of calcium salts and pectin, leading to the waxy structure on the surface of the seed coat, which makes the structure very dense [7].

One of the most attractive features of OCT technology is its capability of providing high-resolution cross-sectional images, revealing the sub-surface internal microstructures of seeds. [8] However, for some seed samples, it was found that OCT has a limited penetration depth; or it cannot see through the seed coat due to the attenuation of backscattering light caused by the compositions of seed coats. Furthermore, for some seed samples, the seed coat layer and cotyledon layer cannot be distinguished by using OCT because their components have similar refractive index values. Therefore, there is a need for a seed coat catalogue to provide essential information about the applicability of OCT technology for investigating seed samples.

Based on the main chemical composition of seed coat and cotyledon ,the seed samples are categorised into four main cultivars: protein-storing, oil-storing, starchy-storing, and carbohydrates-storing seeds. In this work, 14 different crop seeds were selected as the representatives of the four cultivars and measured by SD-OCT. By examining the penetration depths of imaging results, the OCT applicability can be effectively assessed. In addition, we also report the first quantitative evaluation of the thickness of the borlotti bean seed coat and pea seed coat.

II. MATERIALS AND METHODS

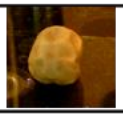
A. Seed Samples And Experimental Conditions

The seed samples used in this measurement were all purchased from Sow Seed Ltd. There are eight different species: broccoli seed, sweetcorn seed, calabrese seed, rapeseed, kohlrabi seed, onion seed, pea seed and borlotti bean seed. Among these seeds, pea seeds and borlotti bean seeds belong to protein-storing seeds; broccoli seeds, calabrese seeds, rapeseeds and kohlrabi seeds belong to oil-storing seeds; sweetcorn seed belongs to starchy-storing seed and onion seeds belong to carbohydrates-storing seeds. The detailed cultivars information of selected seeds, together with their corresponding photos, are shown in Table 1. Five seed samples were chosen for each of the fourteen different seed cultivars. In total, 70 seeds samples were measured, and all measurements were carried out at room temperature (23°C).

B. OCT System Setup

Fig. 1 shows a schematic diagram of a fibre-based spectral-domain OCT used for seed measurement. In brief, a superluminescent diode (SLD) (EXALOS Ltd) was used as the low-coherence light source. This SLD has a centre wavelength of 840nm and a spectral bandwidth full width at half maximum (FWHM) of 50 nm. The light from SLD was coupled into a 2 × 2 fibre coupler where it was equally split into a reference arm and sample arm. The light from the sample arm was focused onto the sample using an adjustable collimator, and the backscattered light from the sample was collected and coupled back to the fibre using the same adjustable collimator. In the reference arm, the light was

TABLE I. PHOTOGRAPHY OF THE SAMPLE SEED WITH CORRESPONDING BOTANICAL NAME

Seed Species	Cultivars	Photography	Seed Species	Cultivars	Photography
Broccoli	*Brassica oleracea L.*		pea	*Pisum sativum Linn.*	
	Brassica oleracea var. capitata f. rubra			*Pisum sativum Macrocarpon Group*	
	Brassica oleracea var. italica		borlotti bean	*Phaseolus vulgaris Cranberry Group*	
Sweet corn	*Zea mays convar. saccharata var. rugosa*			*Phaseolus vulgaris Pinto Group*	
Onion	*Allium cepa L.*		calabresse seed	*Brassica oleracea var. botrytis*	
	Allium cepa 'White onion'			*Brassica oleracea var. gemmifera*	
Kohlrabi	*Brassica oleracea Gongylodes Group*		rape seed	*Brassica napus*	

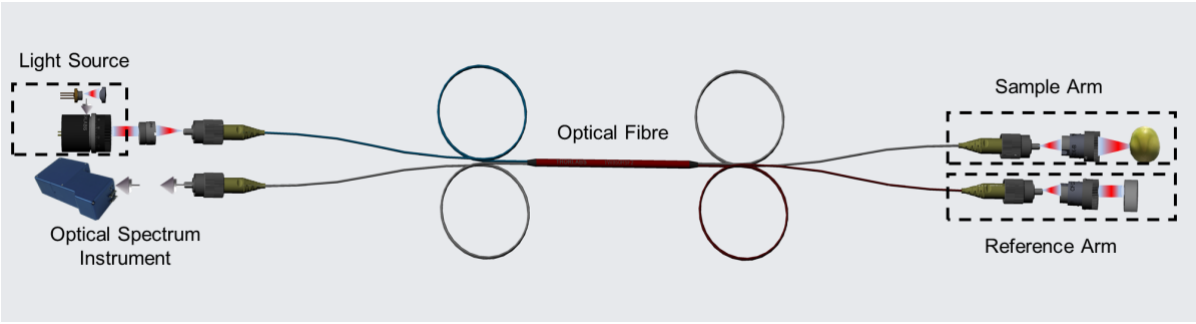


Fig. 1. Schematic of a spectral domain OCT with a pea seed placed at the end of sample arm. The CAD modules of components in this figure are from Thorlabs. Light Source: super luminescent diode, collimator; Optical Fibre: 50:50 wideband single mode fibre coupler; Sample Arm: adjustable collimator; Reference Arm: adjustable collimator, plane mirror; Optical Spectrum Instrument: spectrometer

directed to a plane mirror using an identical collimator as the sample arm. The backscattered and reflected light from the sample and the reference arms were recombined at the 2 × 2 fibre coupler, where the interference takes place. Finally, the interferograms were delivered to a spectrometer (Cobra SRC, Wasatch Photonics Inc.) for detection. The spectrometer has a spectral resolution of 0.04 nm, providing an imaging depth of 2.26mm. The fibre head stage of the sample arm was fixed on a motorised translation (MT S25-Z8, Thorlabs), which can move the fibre probe head at a velocity of 2mm/s. The exposure time of the spectrometer was set as 65 µs. A cross-sectional image (B-scan) consists of 1000 A-scans, covering a length of 8mm across each seed surface. The SD-OCT system has an axial resolution of 9 µm in air, a lateral resolution of 25

μm. The signal to noise ratio (SNR) was measured to be over 95 dB.

C. Quantitative thickness measurement

In the consideration of the applicability of OCT technology, the measurement results can be used to quantitatively measure the thickness of seed coats. Since lower germination speed is witnessed in seeds with less permeable and thicker seed coats, evaluating the thickness of seed coats is crucial in practical settings [9]. Based on this, we further applied image segmentation method[10] for the quantitative analysis of the seed coat thickness. A Canny edge detector was applied to smooth and denoises the B-scans through the Gaussian function firstly. With an adaptive thresholds and sigma values, the Canny detector can help to localize the seed coat area roughly. Then the morphological dilation and erosion operations are performed on the B-scans, a clear structure of seed coat can be obtained. Lastly, two interfaces were marked on the B-scan image to indicate the outer surface and inner surface of seed coat after spline interpolation.

III. Results and Discussions

Fig. 2 shows the acquired B-scan images and the corresponding A-scan waveforms (depth profiles) of the 14 seeds. The air-seed coat interface for all seeds can be clearly observed. Clear seed coat- cotyledon interface has also been observed for the pea seeds (Fig. 2(A), Fig. 2(B)), the borlotti bean seeds (Fig. 2(C), Fig. 2(D)), the sweet corn seed (Fig. 2(E)), and the starchy-storing seeds. As shown in Fig. 2(a)-(e), two peaks can be clear seen in each A-scan, indicating that both the layer of air-seed coat and seed coat-cotyledon can be well distinguished by OCT. Note that all these 5 seeds are the representatives of protein-storing seeds. As shown in Fig. 2(F), the seed coat-cotyledon interface is marginally visible for broccoli seed (one of the oil-storing seeds) which is a sprouting seed after priming processing.

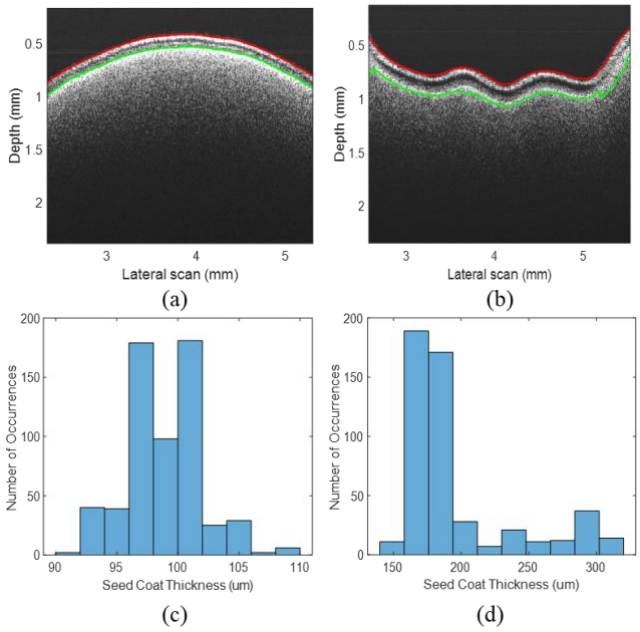


Fig. 3. 3(a) and 3(b) are seed coat segmentation results for borlotti bean and pea seed. The red line and green line indicate the air/seed coat interface and the seed coat-cotyledon. The distance between these two lines indicates the seed coat thickness. 3(c) and 3(d) are the distribution of the seed coat thickness correspondingly.

The seed coat-cotyledon interface is unclear for the other broccoli seeds (Fig. 2(G), Fig. 2(H)), the calabrese seeds (Fig. 2(I), Fig. 2(J)), the kohlrabi seed (Fig. 2(K)) and the rapeseed (Fig. 2(L)). This could be caused by the fact that these seeds all have hard seed coats with abundant oil. However, as shown in Fig.2 (G)-(K), OCT can still penetrate into the seed, and the backscattered light bring back key information about the internal features of these seeds.

Fig. 2 (M) and (N) shows the B-scan image of the cotyledon of two cultivars onion seeds that are representatives of carbohydrates-storing. For these two seed, OCT image only show the near surface features, indicating that OCT cannot penetrate into the seed. There is also one peak in the OCT waveform shown in Fig. 2(m) and (n)), suggesting no backscattering signal underneath the seed coat was detected. Note that the broccoli seed (Fig. 2(H)) was pigmented by green dye, and this dye coating might increase the absorption of the infrared light used in OCT measurement, thus reduce the penetration depth of OCT signal into the seed.

Finally we demonstrate the capability of OCT for quantitative measurement of seed coat thickness. Fig. 3(a) and Fig. 3(b) show the B-scan images of borlotti bean seed and pea seed where the red lines indicate the air-seed coat interface, and the green lines indicate the seed coat-cotyledon interface. Based on this, the distribution of the seed coat thickness could be calculated and shown in Fig. 3(c) and (d). The thickness of borlotti bean and pea seed are 99 ± 3.2μm and 197± 42.3μm, respectively. The obtained thickness of seed coat could be used to dormancy breaking and germination time prediction in the future work.

Conclusion

OCT is a new emerging non-destructive technology for seed evaluation thus it is important to understand the limitation of the technology when imaging seeds. In this work we measured 14 seeds from different cultivars using a fibre based OCT system. Our experimental results suggest that the proposed OCT is highly suitable for the measurement of protein-storing seeds, especially leguminous seeds, such as pea seeds and bean seeds. These seeds have a relative high proportion of protein in the seed ingredient. As for the oil-storing seeds, the seed-coat interface can only be marginally resolved, possibly because of the similar reflex index of seed coat and cotyledon as both contain rich oil content. It was found that the OCT penetration ability depends on the type of seeds, seed coat compositions, and the coating pigment. Furthermore, the seed coat layer thickness can be quantitively measured by applying an image segmentation method to the obtained OCT images. It can be envisioned that OCT would be an effective methodology to investigate the thickness of the seed coat, especially the leguminous seeds, for providing a theoretical basis on seed ingredient and internal structure.

Reference

[1] D. Huang et al., "Optical coherence tomography," science, vol. 254, no. 5035, pp. 1178-1181, 1991.

[2] J. Clements, A. Zvyagin, K. Silva, T. Wanner, D. Sampson, and W. Cowling, "Optical coherence tomography as a novel tool for non‐

destructive measurement of the hull thickness of lupin seeds," Plant Breeding, vol. 123, no. 3, pp. 266-270, 2004.

[3] C. Lee, S.-Y. Lee, J.-Y. Kim, H.-Y. Jung, and J. Kim, "Optical sensing method for screening disease in melon seeds by using optical coherence tomography," Sensors, vol. 11, no. 10, pp. 9467-9477, 2011.

[4] S.-Y. Lee, C. Lee, J. Kim, and H.-Y. Jung, "Application of optical coherence tomography to detect Cucumber green mottle mosaic virus (CGMMV) infected cucumber seed," Horticulture, Environment, and Biotechnology, vol. 53, no. 5, pp. 428-433, 2012.

[5] D. Joshi et al., "Label-free non-invasive classification of rice seeds using optical coherence tomography assisted with deep neural network," Optics & Laser Technology, vol. 137, p. 106861, 2021.

[6] K. Kelly, J. Van Staden, and W. Bell, "Seed coat structure and dormancy," *Plant growth regulation*, vol. 11, no. 3, pp. 201-209, 1992.

[7] J. D. Bewley and M. Black, Seeds: physiology of development and germination. Springer Science & Business Media, 2013.

[8] T. C. Chen et al., "Spectral domain optical coherence tomography: ultra-high speed, ultra-high resolution ophthalmic imaging," Archives of ophthalmology, vol. 123, no. 12, pp. 1715-1720, 2005.

[9] L. Nooden, K. Blakley, and J. Grzybowski, "Control of seed coat thickness and permeability in soybean: a possible adaptation to stress," Plant Physiology, vol. 79, no. 2, pp. 543-545, 1985.

[10] H.-D. Cheng, X. H. Jiang, Y. Sun, and J. Wang, "Color image segmentation: advances and prospects," Pattern recognition, vol. 34, no. 12, pp. 2259-2281, 2001

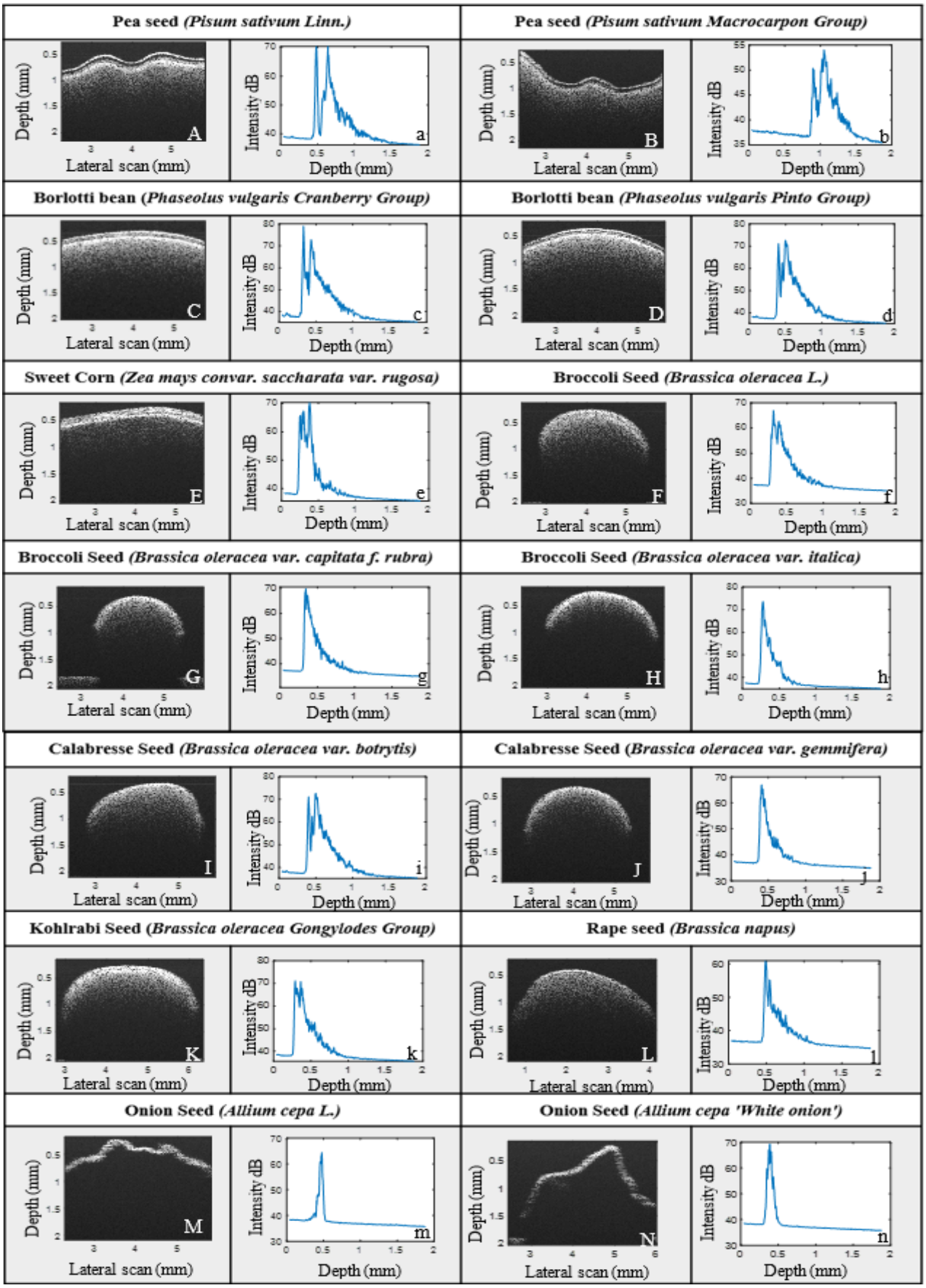


Fig. 2. B-scan images and A-scan waveforms of 14 seeds cultivars. The interface of seed coat-cotyledon can be distinguished in pea seeds (A, B), borlotti bean seeds (C, D), sweet corn seed (E) and broccoli seed (F) with two peaks in their corresponding A-scans. The light can penetrate broccoli seeds (G, H), calabrese seeds (I, J), kohlrabi seed (K) and rape seed (L) but the seed coat-cotyledon interface cannot be observed. The cotyledon is invisible for onion seeds (M, N) as the light cannot penetrate the seed coat.