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Investigating biospeckle laser analysis as a diagnostic method to assess sprouting damage in wheat seeds

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ABSTRACT

Sprouting Damage is a persistent quality control concern in the cereals industry, as sprouting damaged kernels (SDK) contain enzymes that have a detrimental effect on flour quality. Furthermore, the severity of sprouting damage is difficult to detect using standard visual grading methods. In this work, we present Biospeckle Laser Analysis (BLA) as a diagnostic tool to measure the germination progress and the simulated SDK severity of Canadian Western Red Spring wheat seeds. We first analysed dissected seeds and found that high frequency biospeckle activity in the germ correlated with germination progress. Following this, a novel whole seed grading protocol was developed using qualitative and quantitative data provided by the biospeckle measurement. Using our whole seed grading protocol, seeds subjected to simulated SDK treatments at two levels (10 and 20 h pre-trial water exposure) could be differentiated from healthy seeds and from each respective treatment ($p < 0.05$). Our results indicate that BLA has the ability to detect latent SDK and may have further applications such as characterizing the dormancy traits of wheat cultivars and studying the seed germination process.

1. Introduction

The port of Vancouver exports approximately 40,000 tons of grain daily. Grain is loaded onto vessels from portside elevators, which act as holding stations for cereal grains destined for overseas markets. The process of loading grain may take several days, depending on shipment size and weather conditions. Purchasers of grain and government regulators routinely monitor grain quality during the loading process. Samples are taken from the conveyor belt and inspectors visually grade the grain, looking for a myriad of conditions. Of particular importance is the presence of sprouting damaged kernels (SDK). SDK, also known as pre-harvest sprouting damage, are seeds that have germinated in the field before harvest. Such seeds have a substantial negative effect on flour quality.

SDK occurs while the seed is still within the wheat head. Maturing seeds exposed to rain water may germinate within two days following initial imbibition (Mares and Mrva, 2009). Germination induces the production of alpha-amylase, an enzyme that breaks down starch in the endosperm into sugars and ultimately reduces the efficacy of flour as a thickening agent. Additionally, SDK results in protein degradation by increased endoprotease activity (Simsek et al., 2014). The gluten proteins found in wheat are largely responsible for the elastic properties of kneaded bread dough. Degradation of these proteins reduces the ability of bread dough to trap carbon dioxide from the leavening agent and rise properly during the baking process.

Visual grading for sprouting damage is a binary measurement whereby seeds on the conveyor belt are graded as either sprouting damaged or healthy. In grain grading practice, a sprouting damaged kernel is defined as a seed where the radicle has broken out of the seed coat and is visually apparent (USDA, 2004) (Fig. 1). Seed colour is also taken into account, as the hue of the seed coat is an indicator of sprouting resistance (Bassoi and Flintham, 2005). Visual grading of wheat seeds, although a good method to indicate the presence of sprouting damage, does not provide a measurement of SDK severity.

1.1. Biospeckle laser analysis as an alternative to manual grading

Optical techniques can provide a suitable alternative to manual inspection of seed, as they are inherently fast and non-destructive, hence their applicability as a diagnostic tool in the cereal industry is worth exploring. Spectroscopy, which analyses the specimen by colour, has been shown to be a promising wheat seed sorting tool

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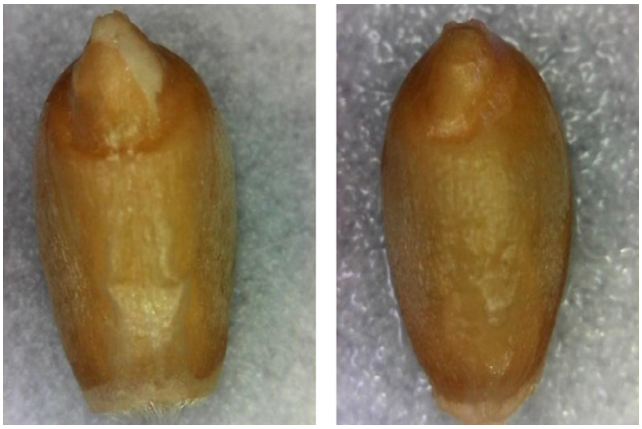

Fig. 1. The radicle emerging from the seed coat in a sprouted seed after 20 h (**left**) and a healthy seed showing no signs of SDK (**right**).

Fig. 2. A seed illuminated with white light (**left**) and monochromatic laser light (**right**). The speckled appearance of the seed illuminated with laser light is a consequence of light interference.

(Vrešak et al., 2016), moisture content sensor (Peiris and Dowell, 2011) and SDK detection method in wheat (Xing et al., 2009, 2010). Magnetic resonance micro-imaging has studied the movement of water in dormant and non-dormant wheat seeds (Rathjen et al., 2009). Machine vision, combined with neural networking, can detect SDK seeds using qualitative features of the kernel (Shrestha et al., 2016) and soft X-ray analysis can differentiate healthy and SDK seed (Neethirajan et al., 2007). The use of laser light offers distinct advantages among optical techniques – laser light technology has the potential to describe biologically active regions of the specimen by measuring light interference variation, providing information otherwise unattainable by human observation or multispectral techniques.

Biospeckle is a laser light technique based on measuring light interference. Lasers provide monochromatic light, thus light interference effects are strong and can be clearly observed under prepared conditions. In the biospeckle technique, monochromatic laser light illuminates the test sample. Variations at the sample surface, such as physical movement or a change in the local refractive index, alter the optical path length that light must traverse. This time variant path length shifts the relative phase within packets of photons arriving at the camera, resulting in a transient interference pattern incident on the image sensor. The observed interference pattern has a grainy texture called 'speckle' (Fig. 2).

Biospeckle techniques were initially studied as a method to monitor processes that involve a change in moisture content or imbibition, such as paint drying and seed viability (Arizaga et al., 1999). Biospeckle measurements are difficult to quantify due to the natural heterogeneity of processes under observation, however previous studies have shown the potential of biospeckle to assess seed viability (Braga et al., 2003), correlate with the presence of fungal infection in beans (Braga et al., 2005), and indicate bruising in fruit (Vega and Torres, 2013). The biospeckle phenomenon has also been assessed by frequency analysis, whereby biospeckle data is decomposed into multiple images that represent speckle variation over a discrete range of frequencies. The germ and endosperm of a maize seed can be differentiated using this approach (Sendra et al., 2005), and biospeckle harmonics, together with the inertia moment metric, have been shown to differentiate between healthy bean seeds and those infected with *F. oxysporum* and *A. flavus* (Rabelo et al., 2011).

In this work, we will illustrate the capabilities of Biospeckle Laser Analysis (BLA) as a diagnostic tool to measure the germination of Canadian Western Red Spring wheat seeds. After establishing a suitable BLA metric to measure germination, we will demonstrate BLA as a novel application of the biospeckle technique by developing a whole seed grading protocol to assess seed germination. By exploiting the temporal and spatial data that is unique to the biospeckle technique, we will show that the BLA method can differentiate simulated SDK seeds from a healthy control group based on SDK severity.

2. Theory – BLA algorithm

The mathematical method chosen to interpret raw data was central to the development of BLA. The LASCA method (He and Briers, 1998) reduced the spatial resolution of the BP, thus it was unsuitable for analysis of an entire seed as morphological changes in the seed were not clear. The Fujii (Fujii et al., 1987) and Generalised Differences methods (Arizaga, 2002) were sensitive to seed discolouration and shadows, causing difficulty in comparing results between seeds and bringing into question the causation of the measurement (Fig. 3). Instead, a Fourier transform approach was chosen to permit frequency analysis of the biospeckle signal. Using this Fourier method, captured image data was restructured into vectors representing a time series of speckle values for each spatial point (pixel) in the captured images. For each vector, the mean was removed by subtraction and a Hamming window was applied. The Hamming window attenuated the vector at each end, improving the periodicity of the signal. Vectors were Fourier transformed, producing the power spectral density (PSD) for each of the spatial points in the 640 × 480 pixel raw image data. This can be represented mathematically, where **I** is a vector consisting of a time series of *N* pixel values at the pixel location *l,m* in the captured image data, and *k* is related to the Fourier probing frequency. First, the mean was removed and a Hamming window was applied to create the zero-mean vector **x** at location *l,m*:

$$\mathbf{x}_{l,m} = Ham \cdot (\mathbf{I}_{l,m} - \bar{I}_{l,m}) \tag{1}$$

Then the Discrete Fourier transform was applied at each pixel location to calculate the frequency content *X* at the frequency described by k:

$$X_{l,m,k} = \sum_{n=1}^{N} x_{l,m,n} \cdot e^{-\frac{i2\pi kn}{N}}, \; k \in \mathbb{Z}, \; 0 > k > \frac{N}{2} \tag{2}$$

The power spectral density is proportional to the square modulus of the Fourier transform *X*, thus we calculated the PSD, *S* as:

$$S_{l,m,k} = \frac{2}{N \cdot f_s} |X_{l,m,k}|^2 \tag{3}$$