Mapping of lignin and compression wood using near infrared hyperspectral imaging in *Pinus radiata* clonal trees

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Introduction

In a forestry research programme in New Zealand, there is a great need for faster wood property mapping techniques to reveal the spatial variation of physical and chemical wood properties within tree stems. The main wood properties of interest to the programme are digital RGB imagery of green wood, microfibril angle pattern maps in 2D by ultrasound velocity, grain orientation by T2 imaging, and chemical composition. The methods need to be high throughput as large numbers of discs (in the order of 1000s) are involved. The wood property data collected will be used to benchmark the simulation of physical processes involved in tree growth, wood formation and their interactions. It was clear that traditional wood chemistry analysis was not a solution to the problem. In addition to being expensive and slow, it only measures the average content of the chemical constituents and yields no information on the spatial variation that exists within a tree. For example, compression wood zones often develop in the down-wind or down-slope side of softwood trees and result in significant variation in chemistry and wood properties within stems.

Near infrared (NIR) spectroscopy is an advanced spectroscopic tool for non-destructive evaluation of chemical properties such as pulp yield, lignin and cellulose content as well as physical properties such as density, microfibril angle and stiffness, using increment cores, solid wood or wood meal[1-3]. This one-dimensional spatial resolution of NIR spectroscopy cannot account for the circumferential variation exhibited by most wood properties due to the heterogeneous nature of wood. Mapping of wood properties such as density and strength using a NIR spectrometer in conjunction with multivariate data analysis have been reported previously. However, the data was collected using a NIR spectrometer fibre optic probe which limits the information only to the specific region presented to the instrument[4].

Hyperspectral imaging is an emerging technique that integrates conventional imaging and spectroscopy to attain both spatial and spectral information from an object. Key advantages of hyperspectral imaging are its ability to obtain spatial information and its non-destructive nature as the sample preparation steps, such as chipping and grinding, are eliminated thus speeding up the process substantially. Use of an imaging spectrograph together with multivariate analysis has been reported as a real time monitoring tool for mapping compression wood and other physical wood properties in pine and spruce[5-7]. However, these studies have utilised mainly the visible spectrum and chemical information was not obtained. Here we describe a novel way of utilising an imaging spectrograph fitted with an NIR camera to obtain NIR hyperspectral data from wood cross-sectional surfaces which are then processed using partial least squares regression to produce two-dimensional maps of lignin in two *Pinus radiata* clones.

Materials and Methods

Samples

Two *Pinus radiata* clones from an 8-year old stand in Esk Forest, Hawke's Bay, New Zealand were used in this study. Five discs per clone (25-30 cm thickness) were cut at 0.5 m, 1.5 m, 6.6 m, 8.6 m and 11.5 m height using a purpose built cutting rig. Disc diameters were in the range 8-35 cm and bark was removed from the discs.

Due to the random nature of light scattering from the rough chain-saw cut surface, the transverse surface of discs was smoothed with a modified Peterson Radial Saw Bench (P & B Engineering Ltd, Rotorua, New Zealand)[8]. The saw operates horizontally and uses a saw blade with a large number of teeth to produce a smooth surface similar to a planed surface. Each disc was barcoded and the transverse surface was photographed in the green condition. The discs were kiln dried under mild conditions to avoid crack and check formation at 60ºC dry bulb and 63ºC wet bulb for 2 weeks. Discs were then conditioned at 25ºC and 65% RH to obtain a final moisture content of 12%.

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Near infrared scanning rig

Conditioned discs were scanned with a Specim ImSpector™ imaging spectrograph fitted with an Vosskuehler NIR 300PSCL line camera with a spectral range of 900–1700 nm. A purpose-built scanning rig with a linear transport table (1 mm step size) was used to present the discs to the line camera (Figure 1). The linear table was driven by a stepper motor which was controlled by a micro-controller. The camera was installed at a fixed focal length offset and the disc was adjusted to the focal plane of the camera using appropriate spacers. A constant distance between the camera and disc meant that radii of the disc could be allocated to the spatial pixels of the camera. The camera focus was adjusted to the plane of the disc face by using a black and white contrast target. Images were corrected using a dark image (for noise correction) and a 100% intensity image. The dark image was acquired by covering the lens with the lens cap and acquiring an image while the 100% intensity image was acquired by recording the image of a teflon strip which covered the whole length of the field of view.

The scanning rig allowed acquisition of NIR image data for a whole disc in approximately 1-2 minutes. For each disc, the spectrograph produced a single frame at 1 mm intervals comprised of 256 wavelengths and 320 line positions.

Figure 1. NIR scanning rig.

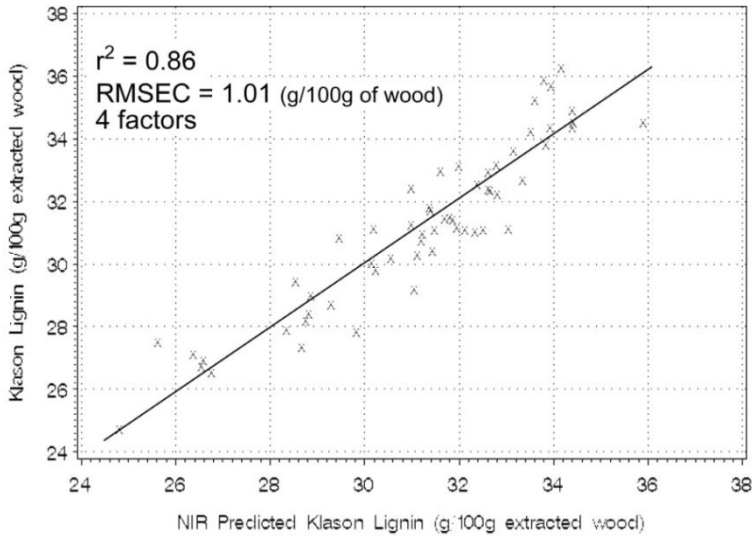

Figure 2. Plot showing NIR/PLS predictions vs Klason lignin values.

Chemistry data

For the model calibration, a set of 20 x 10 mm bark-to-bark diameter strips were prepared from the discs cut at breast height from the same clonal trees. Strips were conditioned to a moisture content of 12% and the transverse surface was scanned on the NIR scanning rig. The significant cost of the wet chemistry assessment of wood meant that only a limited number of chemistry samples were able to be analysed. The sub-samples for wet chemical analysis were carefully selected from the strips in order to provide an appropriate range of Y data for model development. Klason lignin values were obtained by analysing 58 pre-extracted wood samples by the standard method[9].

Multivariate analysis

Hyperspectral data sets obtained were processed using SAS (version 9.1) software. Partial least squares (PLS) regression analysis was used to create models linking the matrices of the pre-processed NIR spectra to the Klason lignin values.

Results and Discussion

The number of PLS factors was decided by cross-validation. This is a method in which one sample is systematically removed from the data set and a model is created from the remaining samples and used to predict the value of the extracted sample. The process is repeated for all of the remaining samples. The model robustness was tested by dividing the samples into 4 random sets (a, b, c and d), building new models for the calibration data sets a and b combined (n=28), and validating on sets c and d (n=30) and vice versa

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(Table 1). The PLS model created with the full data set was used to predict Klason lignin (Figure 2). The plot shows a good correlation (r^2=0.86) for this prediction.

Table 1. Validation of the model data.

	Calibration set			Test set		
	r^2	RMSEC	Factors	r^2	RMSEP	Factors
Cal set (a + b) Test set (c + d)	0.88	0.84	4	0.87	1.27	4
Cal set (c + d) Test set (a + b)	0.89	1.03	5	0.84	1.16	5

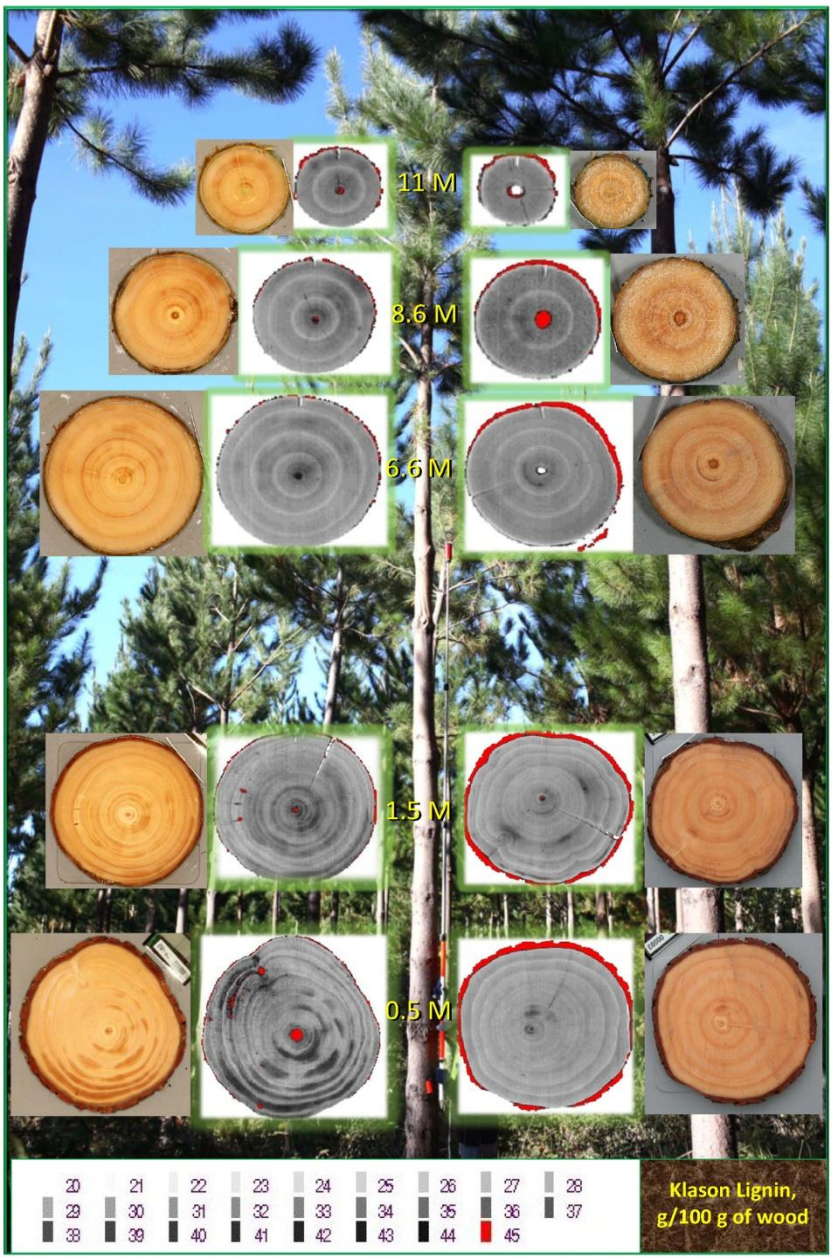

Figure 3. Lignin maps at different stem heights for clones 718 (left) and 819 (right) showing heterogeneous distribution of lignin in clone 718, that is closely linked with the compression wood formation shown by the RGB images. The background picture shows clone 718 in sk Forest, Hawke's Bay, NZ.

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Cross-sectional lignin maps at 1mm^2/pixel resolution were created from the stem base to the top for two *Pinus radiata* clones (Figure 3). The circumferential variation of lignin content in the two clones differed substantially near the stem base. In *Pinus radiata* the normal lignin content usually ranges from 25-28 %; however, with compression wood formation, the lignin content increases to 30-33% in mild cases and up to 38-40 % in severe cases. Clone 718 shows a patchy distribution of high lignin (35-38%) areas compared to clone 819 in which normal pith to bark variation is present. The lignin maps clearly show that large chemical property variations can exist within a tree. Commonly-used techniques, such as taking increment cores, are therefore not representative of the whole tree. Compression wood formation has been linked with chemical indicators such as high lignin and galactose[10]. The spatial distribution of compression wood was mapped by colour scanning the smoothed, wet surface of the discs with an RGB camera. The close correlation between NIR lignin images and the RGB images shows the circumferential variation in lignin is due to heterogeneous compression wood formation in clone 718.

Conclusion

NIR hyperspectral imaging coupled with multivariate data analysis has proven to be a very useful high-throughput method for mapping the heterogeneity of the wood matrix, in this case the chemical composition. Property mapping is a much faster way to reveal the spatial variation of wood chemistry within the tree.

Acknowledgements

This work was funded by Future Forests Research Limited.

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