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Contribution of High Resolution FTIR imaging for the study of changes of wood components during successive ring formation in a tree: links with the microdensitometric profiles



Jean-Paul CHARPENTIER, Kévin ADER and Philippe ROZENBERG (INRA Orléans), Alexandre MICHELET (PerkinElmer France),



Introduction

The successive tree-rings in a tree-stem keep details of the variation of tree response to environment on as many years as the number of rings available. The within-ring variation of wood reflects growth variations of the tree principally linked with the weather variations during the growing season. Thus tree-rings are an original and powerful way to retrospectively study adaptation to climate in forest trees.

The study of tree rings microdensitometry showed that the largest variations in density were at the intra-ring, earlywood (formed in early growing season) to latewood (formed at the end of growing season). The reliation between adaptation and tree-rings is mostly explained by the great change from earlywood to latewood for structural, anatomical and hydraulic properties, associated with the genetic component of between-tree variation.

Therefore, studies of tree response to environment variation and of the consequences on wood production require methods and tools to describe the relevant wood properties at a fine scale, between and within rings. Through the traditional X-ray microdensitometry method (MDM), the density is

Invoign the traditional X-ray microcensitometry method (MUM), the density is measured at very fine scale from a simple increment core. However the method is cumbersome since it requires very precise machining of wood samples to produce X-ray images of these samples. A faster way to implement and provide information with a similar quality would be

A raster way to implement and provide information with a similar quality would b of great interest in research on wood material.

The high resolution Infra Red imaging method was tested for acquiring some images of successive tree-rings especially for wood structure and components and we compared provided informations with the microdensitometry method (MDM) by performing both methods on the same area of the same samples.

This new IR imaging approach could be a very complementary tool to the traditional MDM method to analyse the fine variations of wood structure.

Instrumentation



The system uses a rapid-scan Near and Mid/R spectrometer with a conventional FT-IR microscope. The custom-designed MCT linear detector array has 16 elements. Images are built up by stepping the sample stage. Stepping is synchronised with the interferometer scan, occurring as the scan direction is reversed, with the interferometer scanning continuous). This generates images at rates of up to 170 pixel/siscond. The image size is limited only by memory considerations.

An immediate result of using a linear array is that there is space for connections to each detector element, rather than having to bond to a silicon substrate. This allows photoconductive operation with a wavelength range to 690 cm-1.

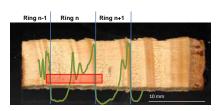
The magnification is variable, to provide pixels that are either 1.56, 6.25, .25 or 50mm across. A single element detector with a range to 600 cm-1 is mounted alongside the array to provide single point measurement. Switching between the image and single point modes simply involves moving the remote apertures of the microscope.

Using a small array reduces possible pixel-to-pixel variation in the images because a flat field and uniform illumination are required only over a small area. It also facilitates obtaining identical background spectra for each detector element.

The imaging system can be used in mid-IR, ca. 6000-690 cm¹, or NIR, 7800-2200cm-1 simply by switching the motorised sources and beamspilters. The outstanding SN performance allows IR reflectance imaging an tingspectral resolution. ATR imaging, also illustrated here, not only extends the range of samples that can be measured, but offers enhanced spatial resolution.

Data collection and results

Analysis is performed on a small board. The microdensitometric profile of this sample has been previously performed. The selected area for IR analysis includes a complete ring with important density variation in the latewcod region. The visible image, NIR scanning area, and microdensitometric profile are overlaid on the image below.



MIRS scan zone

Data Collection NIR Spectra are collected in reflectance mode at 16 cm-1 resolution, 8 accumulations, on the 7800 to 3000 cm-1 range. Pixel size is 25µm x25 µm. Image size is 10mm x 1mm and has 16 000 pixel

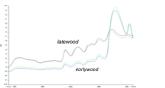
A first image shows the absorbance variation between different areas. Color

indicates the total absorbance of the pixel on the whole spectral range. This absorbance level depends on many factors as for example wood proxisity, surface quality, and of course chemical nature of each point. Those multiple factors affecting total absorbance explain that this view mode is not very selective and doesn't fit perfectly the microdensitometric profile.

Absorbance color scale

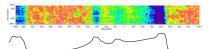
Display on bands selection

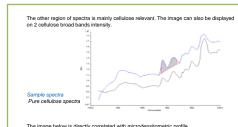
The observation of spectra from a high density area of earlywood and latewood indicates a more intense band in OH absorption region for the earlywood.



The image can be displayed as a chemi-map which displays the intensitity of the selected region.







The image below is directly correlated with microdensitometric profile.

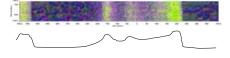
This approach shows how a simple spectral range for image display can help to establish a link between infrared and microdensitometric data.

Statistical approach

A powerful data analysis is available using statistical tools as Principal Component Analysis or SIMCA. Principal Analysis Component

Component

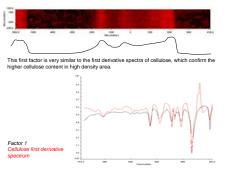
All the data set is processed to extract the main factors from which each spectrum can be reconstructed with a minimum residual. Each spectrum is a mixture of these factors, the image shows how much these factors are present in the spectrum. A color is associated to a factor. The image below shows the intensity of the three most representative factors, calculated after a first derivative.



The factors determined by PCA (Principal Component Analysis) seem to be linked to the density of the wood. The three factors are shown on graph below.



Using the first factor is enough to fit the microdensitometric profile



As many data are available and classified, it is possible to attempt a modelisation of classification for early

wood and latewood. Thirty spectra from each area are extracted from the image and are defined as a

members of class 1 (earlywood) or class 2 (latewood). A data processing (first derivative) is applied to



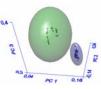
A PCA is processed independently on each class. Two sets of factors are determined, and limits of the residual and scores for each factor are calculated in order to define which criteria a spectrum must match to be classified in Class 1, Class 2 or none.

The 3 dimensional view plots all the spectra in a space where axis are scores 1, 2 and 3. As we see no overlapping between the two classes, the independent modeling seems to be efficient to separate and classify spectra.

SIMCA

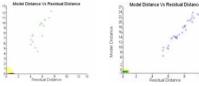
enhance difference between spectra.

An other way to appreciate class separation is to display on the same graph the Class 1 and Class 2 samples, with residual distance to Class 1 on X-axis, and model distance to Class 1 on Y-axis. A second graph is also displayed axis related to Class 2.



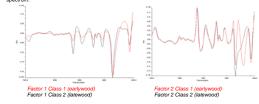
10 11

3-dimensional view of class separation



Class 1 axis, Class 1 points, Class 2 points Class 2 axis, Class 1 points, Class 2 points

With SIMCA, factors are determined independently. So, it's interesting to look at the factors for class 1 VS factors for class 2. The factor 1 is nearly the same for the two classes, it is the cellulose first derivative spectrum.



Factor 2 form Class 1 and Class 2 are similar on the main part of the spectral range, but they are very different on the OH region near 3500 cm-1. It appears clearly that this feature is only present in Class 1 samples. Observing further factors leads to the same conclusion that there is a real chemical difference between earlywood and latewood spectra, and not only a density variation.

To test the capability of the SIMCA method, 50 independent spectra from different parts of the board have been submitted to the model and all have been correctly classified.

Conclusion

This study highlights the capability of NIR imaging enabling the detection of differences in the chemical composition in a tree ring, as well as, correlating accurately and specifically the microdensitometric profile.