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APPLICATIONS MANUAL

BLUE INTENSITY IN *PINUS SYLVESTRIS* TREE RINGS: A MANUAL FOR A NEW PALAEOCLIMATE PROXY

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ABSTRACT

Minimum blue intensity is a reflected light imaging technique that provides an inexpensive, robust and reliable surrogate for maximum latewood density. In this application it was found that temperature reconstructions from resin-extracted samples of *Pinus sylvestris* (L.) from Fennoscandia provide results equivalent to conventional x-ray densitometry. This paper describes the implementation of the blue intensity method using commercially available software and a flat-bed scanner. A calibration procedure is presented that permits results obtained by different laboratories, or using different scanners, to be compared. In addition, the use of carefully prepared and chemically treated 10-mm-diameter cores are explored; suggesting that it may not be necessary to produce thin laths with the rings aligned exactly perpendicular to the measurement surface.

Keywords: Dendrochronology, Scots pine, x-ray density, resin extraction, Fennoscandia.

INTRODUCTION

Knowledge of past climatic and environmental changes can improve our understanding of natural climate variability and address the question of whether contemporary climate change is unique when viewed in a longer-term context (Jansen *et al.* 2007). Compared to many palaeoclimate proxies, tree rings are generally widespread in their distribution, providing information with annual to seasonal resolution. Several long, absolutely dated chronologies exist, which in some cases span over 10,000 years (Friedrich *et al.* 2004) and provide significant potential for the study of past environmental change. Tree rings also provide a wide range of physical and chemical proxies of palaeoclimate, including radial growth, x-ray densitometry, stable isotopes and reflected light image analysis (Briffa *et al.* 2002; McCarroll and Loader 2004; Campbell *et al.* 2007; Grudd 2008; Loader *et al.* 2008).

X-ray densitometry in particular has proven a valuable temperature proxy in coniferous treeline species, and can provide a very strong palaeoclimate signal, particularly in areas where ring widths exhibit little variation (Parker and Henoch 1971). Kirdyanov *et al.* (2008) demonstrated that densitometry measurements from northern timberline larch stands in Eurasia were significantly better correlated with summer temperatures than ring widths. Similarly Cleaveland (1986) used trees from a semi-arid site in the southwestern United States to demonstrate the utility of densitometry measurements to reconstruct drought conditions in an area that traditionally produced relatively complacent ring width series. However, extracting density information from trees has historically been hindered by analytical procedures that were both expensive and time consuming (González and Eckstein 2003; Silkin and Kirdyanov 2003; Sarén *et al.* 2004). In response, research-

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ers have investigated image-analysis techniques as an alternative to traditional x-ray densitometry and recent advances have enabled a renewed evaluation of reflected light image analysis (Sheppard *et al.* 1996; McCarroll *et al.* 2002; Sheppard and Singavarapu 2006; Campbell *et al.* 2007).

The early research using reflected light images investigated the relationship between wood properties related to relative density (*e.g.* cell-wall thickness, cell size) and various environmental variables (Müller-Stoll 1963; Green 1965). Yanosky and Robinove (1986) coupled a personal computer to a video digitizer to measure or 'map' anatomical features within a digitized image of loblolly pine (*Pinus taeda* L.). This process permitted the identification of fibres within annual ring boundaries and the corresponding tree-ring structure. These areas were easily differentiated based on image brightness and the ratio of lumen to cell wall within groups of fibres and was determined to be related to environmental factors (Yanosky and Robinove 1986). However, there are limitations in using this image-analysis method. In particular, the restricted spatial resolution suggests that successive narrow rings would be difficult to distinguish, thus limiting the ability of the imaging system to measure the tree-ring structure in the required detail for the variables to act as a surrogate for x-ray density.

Park and Telewski (1993) conducted a study using video image analysis to measure the relative density of the latewood. They analyzed light transmitted through microtome thin-sections of wood and determined that the maximum percentage of cell-wall area had a significant linear relationship ($p < 0.01$) with x-ray densitometry. Limitations of this system include the requirement for thin sectioning of the tree-ring samples with a microtome. Consequently, the method has not been widely adopted.

Sheppard *et al.* (1996) expanded upon the pioneering use of a video digitizer by Yanosky and Robinove (1986) to explore the use of reflected-light image analysis to measure the brightness of red spruce (*Picea rubens* (Sarg.)) tree rings to reconstruct temperature. Incident white light was used to capture digital images of rings using a charged-couple video camera attached to a compound zoom microscope, permitting the measurement of latewood brightness. They concluded that reflected light image analysis could act as a substitute for x-ray densitometry for reconstructing palaeoclimate from tree rings. However, the method is limited by the need to adjust magnification within cores, which alters the relationship between density and brightness measures. Also, as with the method proposed by Yanosky and Robinove (1986), the image system developed by Sheppard *et al.* (1996) produced low brightness values at the end of cores, requiring a correction.

McCarroll *et al.* (2002) avoided the problems of optics by using a flatbed scanner and a region-growing algorithm (bespoke software) approach to measure the brightness of multiband images (representing the red, green and blue components). They demonstrated a strong correlation ($r = -0.95$; $p < 0.01$) between minimum blue 'reflectance' and maximum latewood density of tree rings from the same pieces of wood. Although the results were promising, they were obtained from very short series, which prevented investigation of signal preservation or climate sensitivity over longer timescales, and the method required the use of specialty image-analysis software.

The inconvenience of using specialty software was circumvented by Campbell *et al.* (2007) who applied the blue 'reflectance' method using images produced on a flat-bed scanner that could be analysed using commercially available software, WinDENDRO™, already used routinely in many dendrochronology laboratories for ring-width measurements and the analysis of x-ray scans. The method was tested successfully using much longer series than McCarroll *et al.* (2002) and the name of the method changed to blue intensity (rather than reflectance). When maximum x-ray density and minimum blue intensity measurements are compared for the same samples using standard techniques, minimum blue intensity displayed near-parallel evolution through time and exhibited an equivalent or slightly higher correlation with summer climate variables. The available evidence suggests that, at least for *Pinus sylvestris*, the most important palaeoclimate archive species in northern Fennoscandia, minimum blue intensity provides a convenient and inexpensive alternative to x-ray densitometry (Campbell *et al.* 2007; McCarroll *et al.* 2011).

The aim of this paper is to describe in detail the blue intensity method, to further the application and testing of the method on other important archive species. A calibration procedure is proposed, that enables the results obtained by different laboratories, or using different scanners, to be compared. To date, all published examples of the method have used the carefully prepared and chemically treated 'laths' required for x-ray densitometry. Preparing such laths is difficult and requires specialized equipment, so some experiments are presented to test whether the blue intensity method can be applied directly to 10-mm cores obtained from increment borers.

SYSTEM DESCRIPTION OF BLUE INTENSITY USING WINDENDRO

This section describes the application of the technique using the WinDENDRO^{TM} (hereafter WinDendro) software package, which utilizes semi-automatic image-analysis software and a high definition optical scanner to measure tree-ring width and density variables (WinDendro 2004c, 2008a manual). For ring-width (TRW) measurements, sanded cores or discs are scanned (>600 dpi (dots per inch) resolution) and the images are saved as TIFF (tagged information file format) files. For maximum density (MXD) measurements, it is the x-ray images produced from the laths rather than the wood samples directly, which are scanned and analysed for changes in their grey scale using the more complex 'density version' of WinDendro. A cellulose acetate calibration wedge of known density and with steps of known thickness is exposed to x-rays at the same time as the wood samples and included in the scanned image. This allows the light intensities of the grey level values to be translated into density units (g cm^{-3}). The WinDendro software measures the selected tree-ring parameters (TRW, MXD) using a line of sensors traced along the imported image profile. The measurement path is selected to detect each ring boundary at right angles to the direction of measurement

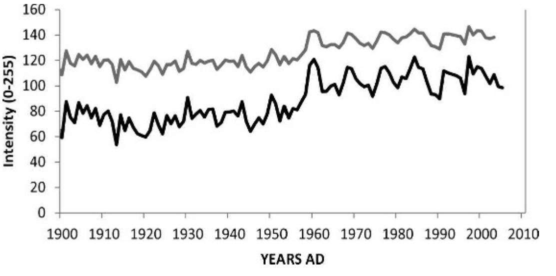


Figure 1. Example of non-calibrated (grey line) and calibrated (black line) blue intensity chronologies (20 trees).

from which profiles of earlywood and latewood ring widths, maximum and minimum density and the mean densities of the earlywood, latewood and of the whole ring may be developed. It is necessary to visually verify the individual tree-ring boundaries, and identify gaps and cracks in damaged samples before saving data files.

When WinDendro (density version) is used to measure blue intensity, the wood samples are scanned as 24/48-bit color images at a resolution of 1,000 dpi and saved as TIFF files. The Swansea University system comprises an Epson Expression 1,680 flatbed Pro Series scanner and SilverFast Ai professional scan software. The first task is to calibrate the scanner so that results are comparable between laboratories using different scanners and are also stable over time. Temporal stability in the power or intensity of the light source cannot be assumed, even where the same scanner is used, because bulbs tend to fade over time, leading to a potential drift in blue intensity values (Figure 1). In the example of x-ray densitometry, calibration is achieved by the x-ray analysis of a wedge with several steps of known density. For the measurement of blue intensity the wedge is replaced by a color card with steps of known blue intensity. The Swansea University laboratory uses a Monaco EZ-color card (monr2004:08-01 version 2). Calibration (adjusting for light source stability) is necessary to ensure comparability of blue intensity values measured at different times or with different equipment.

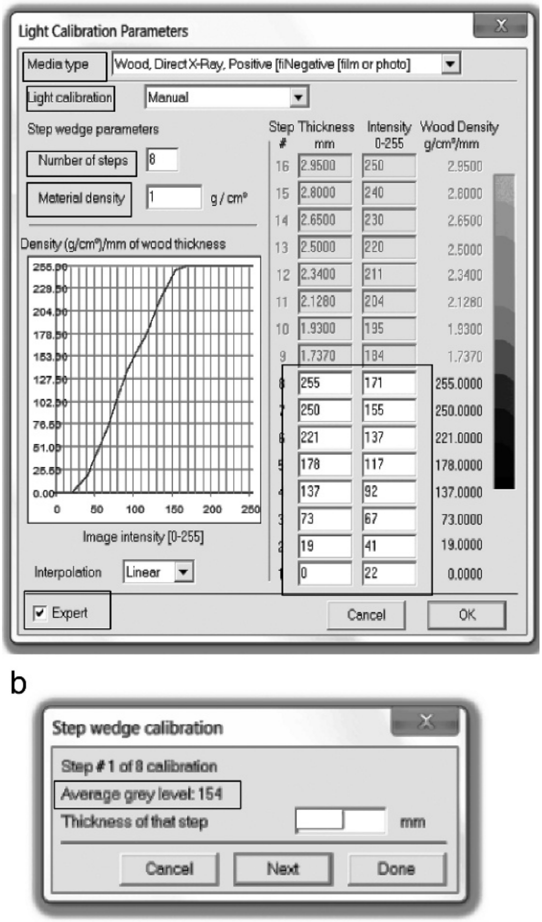
BLUE INTENSITY EXPERIMENTAL PROCEDURES

Here the steps required for analysis of blue intensity on prepared wood samples are described (see sample preparation for description of wood preparation). A step-by-step guide for analysing blue intensity is presented; note that WinDendro commands are grouped in menus along the top of the image frame, for clarity the menu name/ command name will be referred to for specific instructions, *i.e.* "Save Image" command of the "Image menu" was written Image/Save Image.

Calibration Steps

- Using the scanner and SilverFast Ai professional scan software, scan the Monaco EZ-color card (monr2004:08-01 version 2) at 1,000 dpi and save as a TIFF file.
- Convert the raw scan to a 'blue tone' using Display/Channel/Blue command in WinDendro.
- On the top of the menu bar use 'Density/Light Calibration Parameters' to open the light calibration window (Figure 2a).
- Change the 'Media type' to Wood, Direct X-Ray, Positive [Negative [film or photo]].
- For 'Light calibration' select manual for 2008 version of WinDendro or set 'steps detection' to, manual [click each step] for 2004 version of WinDendro.
- Activate 'expert' to edit the light intensity steps. This is necessary to modify the 'Intensity 0-255' column for input of color card measurements.
- Under 'Step wedge parameters' change 'Number of steps' to 8 (eight 'colours' selected for calibration) and 'Material density' is set to 1 g.cm^{-3}. Press 'OK'.
- Along the top menu bar select the 'Density/ start light calibration' command to open a new window that displays a 'Step wedge calibration (Figure 2b) window (WinDendro 2004c manual).
- For the step wedge calibration, the user places the cursor on the scanned color card image for each of the eight 'X,Y' coordinates, 'steps' identified in Table 1. The average 'grey' level (blue tones for the blue intensity and grey values for MXD) is calculated for each of the X,Y coordinates (Table 1) in this window and this value is recorded for manual input into the 'Light Calibration Parameters' window in WinDendro (Figure 2a). This process is repeated five times and the mean values calculated for input in the next step.
- The 'grey' values for each of the X,Y coordinates are manually inserted into the 'Step thickness' and 'Intensity' columns (Figure 2a). The values inserted into the 'Step thickness' column are the original color card values associated with the color card. The values inserted into the 'Intensity column' are the values obtained from the current measurements. This calibration procedure is repeated for each new scanning session.

a



Figures 2. (a) Light calibration parameters window used in WinDendro™ to activate light intensity calibration. The highlighted sections are manually edited by the user. (b) Step wedge calibration window (WinDendro™ 2004c manual). The measure in the highlighted area is recorded for manual input into the Light Calibration Parameters window.

Table 1. The X,Y coordinates for calibration of minimum blue intensity using the Monaco EZ-color card (monr2004:08-01 version 2).

Step	1	2	3	4	5	6	7	8
X	Black	J	G	J	G	D	A	White
Y	Black	22	22	19	19	19	19	White
Step thickness	0	19	73	137	178	221	250	254

Sample Measurement

Having calibrated the scanner, the wood samples, prepared as for ring width measurements (*i.e.* samples surfaced by polishing with progressively finer grit abrasive paper (80 to 600 grit) to enhance ring boundaries), are then scanned and the results saved as TIFF files. Minimum blue intensity is measured using the following procedure.

- Using WinDendro acquire the previously saved TIFF file, and activate the Density/Activate Density command.
- Use the Density/Media type command to select 'Wood'.
- The Data/Ring Based Format command permits the selection of variables for measurement, including ring width and minimum density (proxy for minimum blue intensity).
- Using Display/Channel select the blue channel.
- Using Path/Creation Parameters change the path width to 1 mm and select multi-segments paths.
- To select ring path for analysis click the pith end of the sample. When the Identification window opens enter the sample identifiers and click OK (see WinDendro manual for full *measurement* instructions). The software automatically detects ring boundaries and requests file location to save measurement information.
- Verify the ring orientation and ring boundary placement and repeat the process twice more to give three measurement paths from which a sample mean series is calculated.

Sample Preparation

In the measurement of x-ray densitometry, the clarity of the x-ray image is determined by the the accuracy of collection and sample preparation (Schweingruber 1996). Established protocols include preparing laths using specialized twin-bladed saws (*e.g.* Dendrocut, 2003 from Walesch Electronics, Switzerland). Laths of *ca.* 1.25 mm are considered the optimal thickness (Schweingruber 1988). Also important is the removal of organic extractives, collectively known as resins, commonly found in softwoods. These resins are variable across tree rings, differ between the heartwood and sapwood and marginally influence the volume potentially causing errors in x-ray density measurements (Schweingruber *et al.* 1988; Lindeberg 2004). In the development of the minimum blue intensity technique it was necessary to investigate the influence of this entire sample preparation process on minimum blue intensity measurements.

Previous research (Campbell *et al.* 2007) utilized laths prepared according to the protocols of Schweingruber *et al.* (1988). In this comparison of methods, both x-ray densitometry and minimum blue intensity used Scots pine laths cut to uniform thickness (*ca.* 1.25 mm) and refluxed in ethanol (99.5%) in a Soxhlet extractor for approximately 20 hours. This allowed for a direct comparison of both x-ray densitometry and minimum blue intensity measurements on the same thin laths of wood (Campbell *et al.* 2007). However as minimum blue intensity measurements use reflected light (*i.e.* a scanner) rather than transmitted energy (x-rays), it was decided to explore the use of increment cores (10-mm diameter) as possible alternatives for the thin-sectioned laths used traditionally in x-ray densitometry measurements.

Cores (two per tree) were collected from seven Scots pine (*Pinus sylvestris*) trees located in the Siljansfors Experimental Forest, near Mora, Sweden (60°52′N, 14°19′E). In order to enable a comparison of laths and cores, one of the samples was prepared according to the protocols of

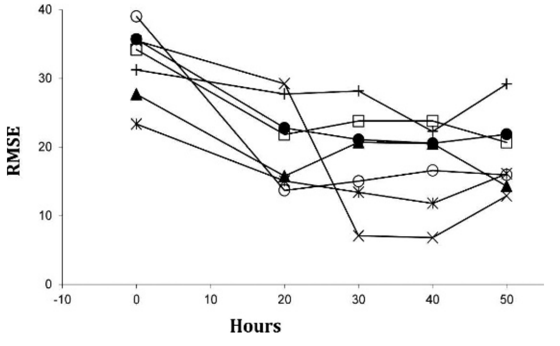


Figure 3. Root mean square error (RMSE) comparing the relationship between the 20, 30, 40, and 50 hour treatments for all seven cores (□) M32, (Δ) M33, (X) M38, (*) M39, (●) M43, (+)M44, (○) M45).

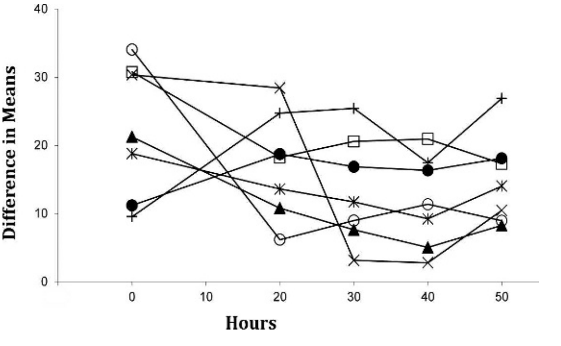


Figure 4. Difference in the means between the 20, 30, 40, and 50 hour treatments for all seven cores (□) M32, (Δ) M33, (x) M38, (*) M39, (●) M43, (+) M44, (○) M45).

Schweingruber *et al.* (1988). Laths 1.25-mm thick were cut perpendicular to the direction of the fibres using a mechanical twin blade saw (Dendrocut, Walesch Electronics, Switzerland). The laths were refluxed in ethanol (99.5%) using a Soxhlet apparatus for 20 hours, to remove resins (Schweingruber *et al.* 1988). To prepare the surfaces for image analysis, the visibility of the annual ring boundaries was enhanced by polishing the samples with fine grit abrasive paper (600 grit). Annual blue intensity measures were obtained from the laths as described above.

The second core was initially prepared according to traditional ring-width measurement protocols. The cores were surfaced by polishing with progressively finer grit abrasive paper (80 to 600 grit) to enhance ring boundaries and produce a finely polished surface. To test the amount of time required to remove the organic extractives, the seven cores were refluxed in ethanol (99.5%) in a Soxhlet extractor for five time periods (0, 20, 30, 40, 50 hours) and air dried in a fume cupboard between treatments. After each of the time periods (0, 20, 30, 40, 50 hours) in the Soxhlet extractor, minimum blue intensity measurements were obtained for each of the cores using standard techniques (Campbell 2009).

The raw (non-detrended) minimum blue intensity measurements for each time period (0, 20, 30, 40, 50 hours) were compared with the lath data (not presented here), none of the treatments clearly demonstrated results sufficiently similar to be acceptable for palaeoclimate analysis. However, in tree-ring research particularly when dealing with ring widths and MXD data, it is the standardized or de-trended series that provide the best climate information. The minimum blue intensity chronologies for each time period (0, 20, 30, 40, 50 hours) and the laths were de-trended using linear regression and standardized to indices by subtraction (using ARSTAN; Cook and Holmes, 1986). The minimum blue intensity results obtained from the laths and cores of each tree are compared using the Root Mean Squared Error (RMSE; Figure 3) and difference of means test (Figure 4). Both the RMSE and the difference of means test suggest that ethanol extraction of cores for 30 to 40 hours produces results most similar to those from laths prepared using established techniques (Figures 3 and 4). When the standardized results of the seven trees are combined, the correlation between laths and cores refluxed for between 30 and 40 hours is >0.9 ($p < 0.05$). The duration of resin extraction described here for Scots pine may not be appropriate for all species/sites. We therefore recommend that when commencing work with other archive species, the nature and duration of this resin extraction step should be evaluated prior to measurement.

The previously described cores and laths were also measured to determine whether blue intensity values are influenced by ring-angle orientations other than perpendicular (90°). This is a critical constraint in x-ray densitometry because even small variations in fibre angle from 90° result in a "smearing" of the x-ray image (Schweingruber

1996; Bergsten *et al.* 2001). The blue intensity technique measures light reflected off the surface, rather than energy passing through the sample, so it is therefore less likely to be sensitive to variations in ring angle. In the laths used, all of the rings were aligned perpendicular to the surface (*i.e.* 90°), however ring angle in the cores varied between 60° and 120°. The Pearson correlation coefficient between laths and cores was $r = 0.95$ ($p < 0.05$) indicating that ring-angle orientation does not affect image-analysis measurements (results not included). The close similarity between the lath and core samples suggest that 10-mm cores prepared as for tree-ring width measurement, but refluxed in ethanol for between 30 and 40 hours to remove the near-surface resins, produce a blue intensity chronology that is highly similar to that obtained using laths prepared for conventional x-ray densitometry. It is therefore not necessary to obtain cores with the rings aligned exactly perpendicular to the measurement surface.

Conclusions

Traditional x-ray densitometry is a powerful but expensive technique, requiring specialist equipment and carefully prepared and chemically treated laths of wood with the rings aligned exactly perpendicular to the measurement surface. Minimum blue intensity provides a much simpler and less expensive alternative approach to obtaining equivalent information for the species studied. Although laths can be used, it was found that 10-mm increment cores prepared as for tree-ring width measurement, but refluxed in ethanol for 30 to 40 hours to remove near-surface resins, produce results equivalent to those produced by x-ray densitometry. Because the method relies on reflected light, the ring boundaries do not need to be aligned perpendicular (90°) to the measurement surface, but may vary as much as 30°. This may permit the use of species such as *Podocarpus* spp. and *Juniperus* spp. traditionally seen as difficult to measure using conventional x-ray densitometry methods. A color card calibration procedure ensures comparability between laboratories and over-time, and permits the application of standardization techniques to improve the preservation of the long-term variability in tree-ring data.

The method requires only a good quality flat-bed scanner and the images can be analysed using the density version of the WinDendro software already used in many dendrochronology laboratories. Other image-analysis software (*e.g.* Coo-Recorder) are also available and if calibrated accordingly may be able to provide results equivalent to those obtained using WinDendro. The method has been used to extract a palaeo-temperature record from Scots pine tree rings in Fennoscandia, and the results are at least as good as those obtained using conventional densitometry (Campbell *et al.* 2007; McCarroll *et al.* 2011). There is great potential for the wider application of this technique to other species and environments.

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