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Effect of sample preparation and scanning resolution on the Blue Reflectance of *Picea abies*

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Introduction

Assessing past and recent climate variability is an important task in environmental change research. Various methods and techniques have been developed in order to generate high-quality proxy data. In Tree-rings, which are perhaps the most important annually resolved proxy that spans the past millennium (Esper et al. 2004), particularly strong climate signals have been achieved by measuring maximum latewood density (MXD) using the widely established X-ray method (Polge 1966, Schweingruber 1988, Parker et al. 1978, D'Arrigo et al. 2000, Cown et al. 2004). Such data have been produced to successfully reconstruct past temperature variations from far northern (Briffa et al. 2001) and high elevation (Büntgen et al. 2006) environments, based on various conifer species. In praxis, the application of this technique is not directly accessible for many researchers due to costly equipment, a laborious sample preparation and time consuming measurement procedures. For this reason, efforts have recently been made to either increase the efficiency of X-ray densitometry (Bergsten et al. 2001) or develop alternative paleoclimate proxies which are as reliable as the X-ray based MXD method, but can be applied more readily and efficiently (Frank 1998, Sheppard et al. 1996, Schinker et al. 2003).

McCarroll et al. (2002) assessed the suitability of multiband digital images (RGB) of pine (*Pinus sylvestris)* laths as a surrogate for x-ray measured MXD. The colour images were acquired using a common flatbed scanner and commercially available software. The blue channel proved to correlate most strongly (r = -0.96) with wood density. Following this study, a direct comparison of the two methods was carried out in building a well-replicated pine chronology (1777-2002) that revealed inter-series correlations of the blue intensity measurements similar to classical MXD data -- ranging from 0.61 to 0.75. Correlation analyses with mean monthly summer temperatures were found to be even higher (-0.65 to -0.80) than those obtained for MXD. This knowledge suggests that minimum blue reflectance (MBR) could serve as a surrogate for classical MXD measurements (Campbell et al. 2007). Since MBR is a relatively new approach, few protocols on wood preparation, methodological proceeding and technique fine-tuning exist. No standard procedure has been established yet and various research questions related to sample surface treatment, scanning resolution, measurement tracks and digital imaging remain open.

The present study investigates for the first time the effect of sample surface preparation and other potential influences such as scanning resolution or image saturation on the quality of the annually resolved MBR measurements. This task seems crucial to standardize the preparation procedures and thus make MBR results from different laboratories directly comparable. For the purpose of this study, a series of laths from a young spruce (*Picea abies*) with relatively wide rings has been produced and both, MXD and MBR were subsequently measured, the latter considering several different sample treatments.

Materials and Methods

Sample preparation

In order to obtain directly comparable results from both, the X-ray densitometry and the reflected blue light measurements, a series of 30 wood laths was produced. These pieces originate from one spruce tree which grew in the area of Birmensdorf in the Swiss lowlands at 47°21′15.98″N, 8°26′16″E, ~500m above sea level. The material was chosen to test the MBR method on a different conifer species than previous studies and the wide rings represent an ideal test bed to assess uncertainty introduced by method. Also does *Picea abies* not include a change in the surface colour based on heartwood/sapwood transition. In a first step, all samples were prepared for density measurements according to the principles described by Schweingruber et al. 1978.

In a previous test using *Pinus uncinata* laths from the Spanish Pyrenees (see Büntgen et al. 2008 for site description), the reflected blue light proved to react extremely sensitively to minor changes in surface colour caused by small cracks, scratches or inclusions. Therefore it appeared necessary to ensure an identical measurement path for the comparison of both methods, MXD and MBR. This was done by scratching a straight track into the surface of each lath, using a sharp needle. This way, the resulting path was visible on the X-ray film (required for conventional density measurement) as well as in the RGB colour-scan which forms the basis of the MBR technique.

To investigate the influence of surface preparation on resulting BR curves, different treatment were applied on a second series of samples at different scanning resolutions. For the first run of scans, the wood pieces were left in the state of a rough cut with a single-bladed saw. Subsequent scans were taken from an ever smoother surface achieved by sanding with grits of 60, 120, 220 and 400 grains per mm^2 ensuring that the wood did not burn as this would bias the result dramatically. To prevent a loss of measurement tracks through sanding, foils were printed beforehand that allowed redrawing the exact tracks after each sanding step.

Measurements

MXD preparation and measurements were carried out at the Swiss Federal Research Institute WSL in Birmensdorf. The data was produced using a WALESCH 2003 X-ray densitometer with a resolution of 0.01mm and brightness variations transferred into g/cm3 using a calibration wedge (Eschbach et al. 1995). Relationships between the absolute (volume and weight) and radiographic (X-ray) wood density (considering different species) were used as correction factors (Lenz et al. 1976). The resulting MXD values are simple measurements of cellulose acetate that can differ from the volumetric-gravimetric value (Schweingruber et al. 1978).

In this study, we used a calibration wedge made from cellulose acetate with a density of 1.274 g/cc and a continuously graded thickness (Schweingruber 1988). For conventional density measurement, cracks and inclusions are avoided if possible, which results in an erratic path that cannot easily be reproduced (Eschbach et al. 1995). For the purpose of this study, a reproducible track is indispensable to ensure the comparability of the two methods. Therefore, the paths which were scratched in advance were strictly followed during the measurement process and superficial disturbances were not bypassed. This has the advantage that the changes in density and light reflection caused by such disturbances remain comparable.

The same samples as used for X-ray densitometry were scanned using an Epson Expression 10000XL flatbed scanner. The hardware resolution of this system is 2400 dpi. It is possible to achieve resolutions up to 4800 dpi in which case an interpolation algorithm is executed. Image analyses were performed using the Image-Pro Plus 4.5 digital software. This program allows individual extraction of the three colour channels (R, G, and B) along a defined straight path to export for statistical analyses and presentation. The segment along which the blue values were taken is 0.08 mm wide. This is narrow in comparison to the 0.14 mm segment length of one of eight possible Dendro 2003 sensors (Eschbach et al. 1995). As a consequence, one single path of BR values is more susceptible to surface variability than an MXD track which integrates a greater area. A median of three parallel BR paths proved to resemble the MXD values best and were therefore considered for the analysis.

Results and Discussion

Influence of surface preparation

Sample surface treatment greatly influences the resulting BR values. The first scans of the roughly cut surface produced a very unsteady curve. Despite the fact that the ring borders could easily be detected with the naked eye, the data showed no decrease of the BR in the latewood and a clear ring pattern remained absent. Figure 1 and figure 2 show the effect of an ever smoother surface on the blue intensity. In table 1, the effect of sanding with different sandpaper grits is summarized. These figures reveal that a very smooth surface is required to gain realistic results with distinct latewood minima. The degree of surface preparation implies sensitivity of the reflectance level to the fineness of sanding.

Table 1: Effect of an increasingly smooth surface on blue intensity data. The degree of graining refers to the different steps of sanding that were applied.

Sandpaper grit [grains / mm^2]	**Effect on resulting BR curve**
60	Some peaks become visible; large variability in the early-wood; pattern unsteady and insufficient for ring identification
120	Peaks become more distinct; early-wood variability is reduced though still noticeable; ring borders are visible
220	BR latewood peaks are even more pronounced; early-wood noise reduced; ring borders distinct; clear trend towards a lower reflectance
400	Noise is negligible; further slight shift towards less intensity; resemblance with MXD curve

Scanning resolution

Comparison of data derived from wood pieces scanned at 600, 1200m and 2600 dpi revealed little difference between the two lower resolutions. Some latewood peaks were more pronounced at 1200 dpi than at 600 dpi, and the variance in the earlywood appeared slightly smaller. If the scanning resolution was increased to 2600 dpi -- which is above the hardware resolution of the device -- the effect on the blue intensity was distinct. There was a strong shift towards less intensity and the peaks of the latewood even reached a BR of zero. The exact reason for this effect is unclear, but it probably results from the interpolation algorithm which is applied to raise the spatial resolution. Thus, the scanning resolution has to be kept within the hardware resolution of the available scanning device to prevent unwanted data manipulation.

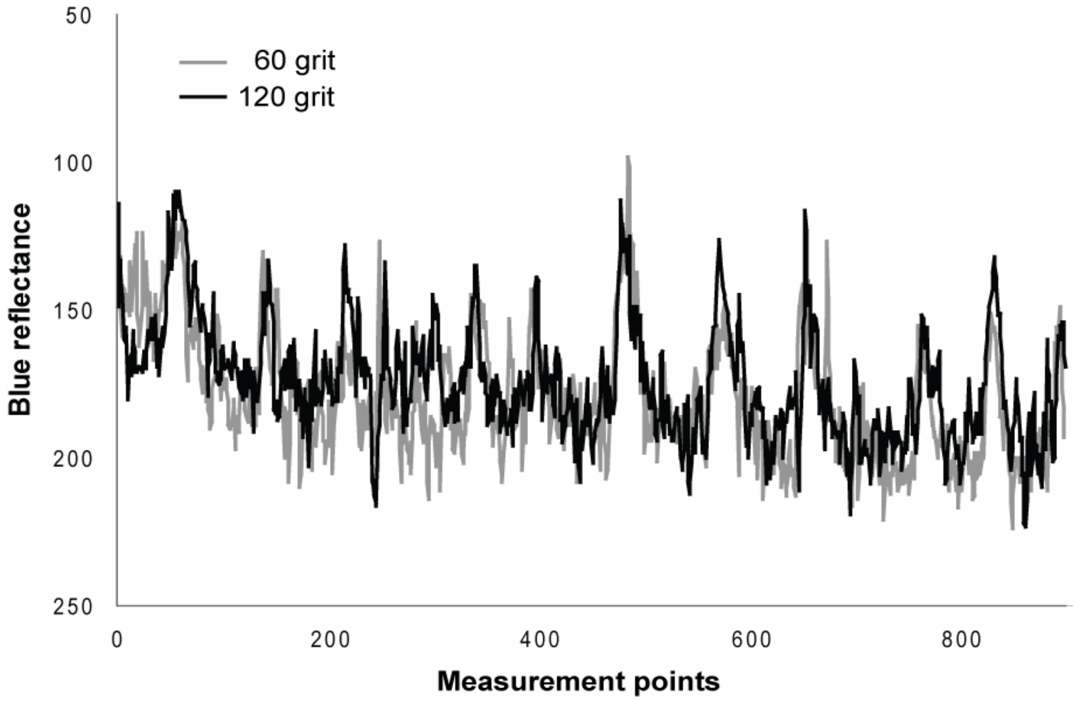

Figure 1: Blue reflectance of a Picea abies sample from Birmensdorf. Grey and black curves show results from scans of surfaces sanded with a 60 and 120 grit, respectively.

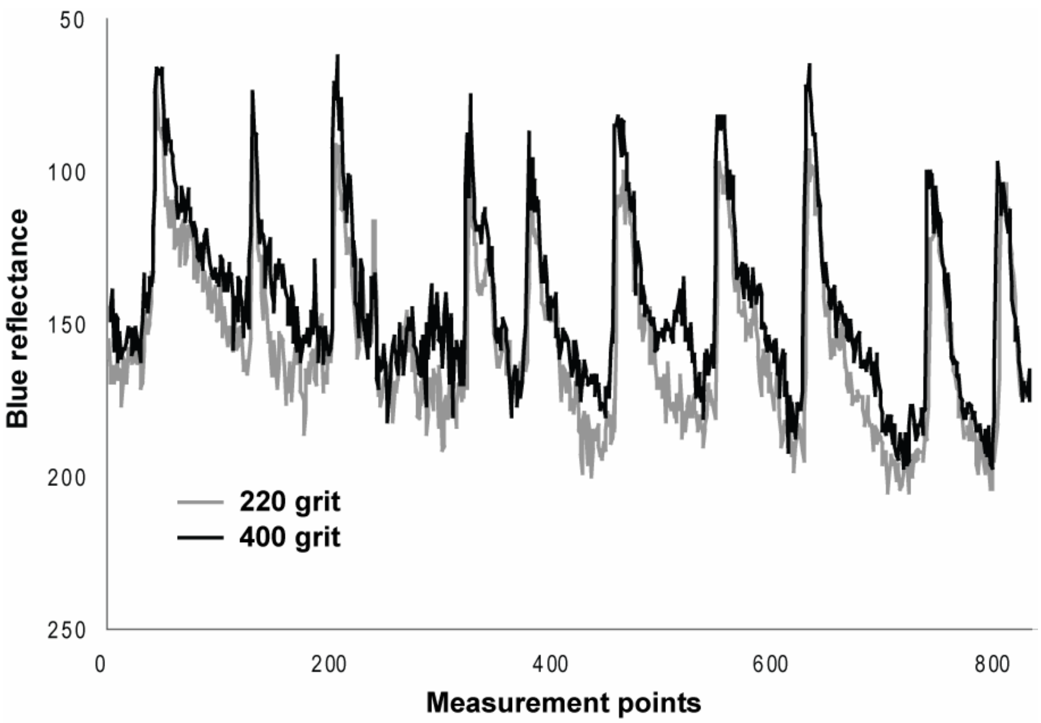

Figure 2: Blue reflectance of Picea abies sanded using a 220 (grey curve) and 400 grit (black) paper .

Colour saturation

The fact that the source of the reflected blue light values is a standard RGB picture allows data manipulation with digital imaging techniques. If the saturation of the digital image is changed, there is a direct influence on the level of the BR curve. Figure 3 demonstrates the effect of a saturation reduction of 50%. The data shown in this plot is obtained from one of the 2600 dpi scans of the *Picea abies* laths in a state of density measurement preparation. This biased data was used to test, whether a change in colour saturation allows the “lost” latewood peaks to be restored and thus a resolution above hardware properties of the scanning device could still be used. When the saturation is reduced, a clear shift towards higher blue intensity is visible and the latewood peaks,are restored. At the same time the amplitude of the peaks is largely reduced. Therefore the saturation of the digital image from which the blue intensity is taken should remain at 100%. This is feasible if an appropriate scanning resolution is considered.

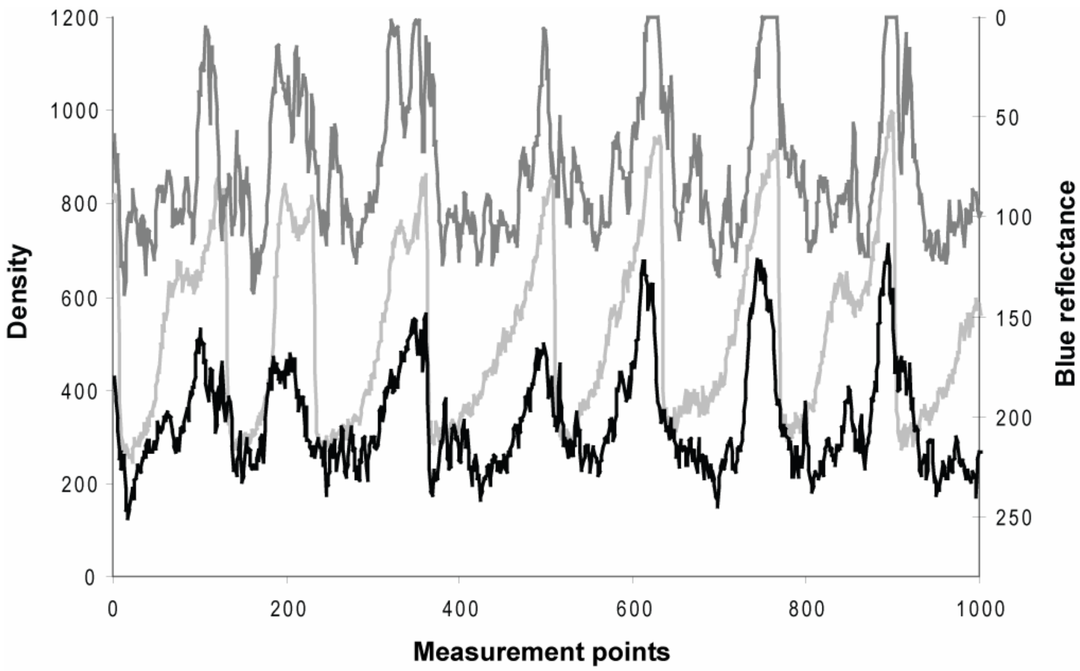

Figure 3: Reduction of the image saturation by 50% of a Picea abies sample. The top curve is the blue reflectance scanned with 2600 dpi at a saturation level of 100%. The middle curve is MXD from the same sample. The bottom curve shows BR with a saturation of 50%.

Comparison of minimum blue reflectance (MBR) and maximum latewood density (MXD)

Previous studies on this subject used *Pinus sylvestris* samples from northern Scandinavia (McCarroll 2002, Campbell 2007). Here, a comparison of the two methods applied on the above mentioned *Picea abies* wood is presented with the aim of confirming the promising results of previous studies using a different coniferous tree species. Despite the finding that a smoother surface improves the BR output, the surface of the wood laths received no further treatment after the wood density had been measured. This was done in order to not artificially alter the direct comparison shown in figure 4. The diagram demonstrates that the data from both methods correlate very well and that apart from the variance in the BR earlywood data, which is reltated to the slightly rough surface caused by the twin-bladed saw, the pattern is quite similar.

Analysis of the density profiles showed that the clearest climate signal derives from the variation in maximum latewood density (Schweingruber 1978). Therefore the crucial question is whether the difference in the minimum blue reflectance (MBR) of each ring reflects the MXD peaks. Figure 5 reveals the analogy of MBR and MXD in 18 consecutive years. The correlation between the two curves is -0.79. The correlation coefficients between MBR and MXD for each of the 30 samples range from -0.56 to -0.89 with an average of -0.78. It is probable that these values could be improved by further smoothing of the surface before measuring MBR.

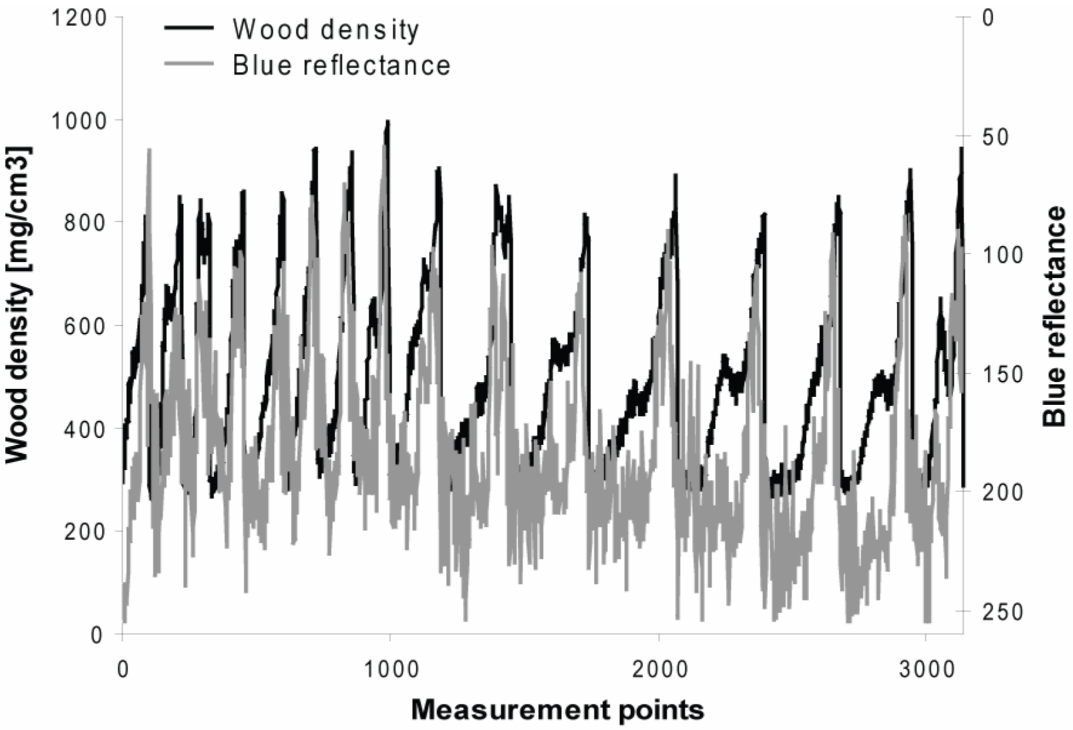

Figure 4: Direct comparison between density and blue reflectance measurements. The surface was treated identically for both methods and a scanning resolution of 1200 dpi was chosen for BR measurements.

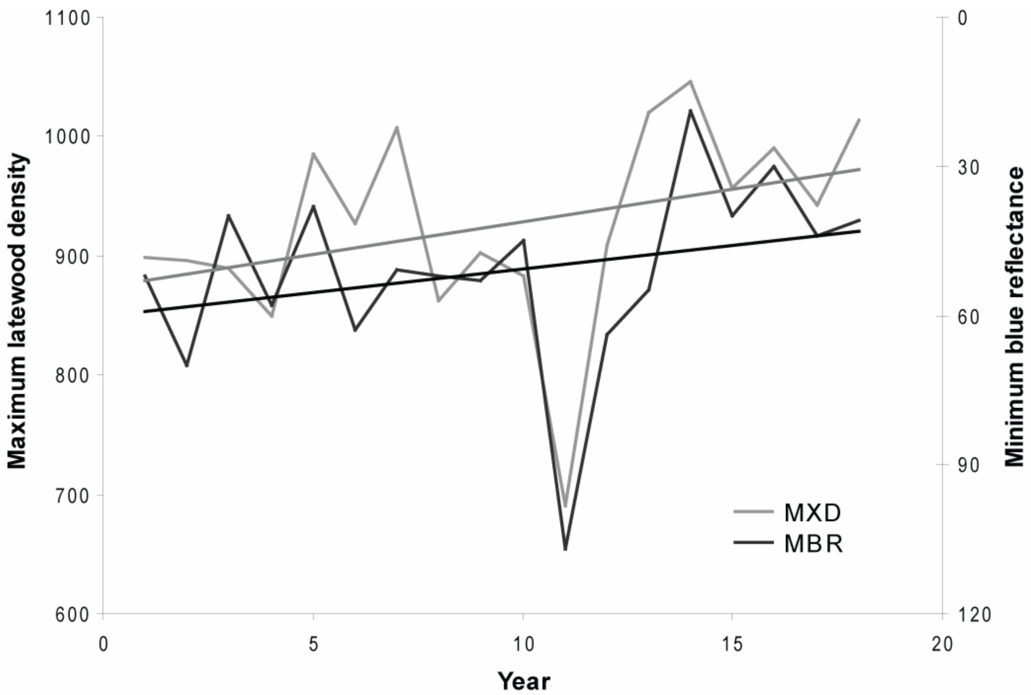

Figure 5: Comparison of minimum blue reflectance and maximum latewood density of 18 consecutive years. Both curves are shown with their linear trends. The correlation between MBR and MXD is -0.79.

Conclusions

Our results emphasize the influence of sample preparation and scanning resolution on the output of blue intensity measurements. It is shown that before Minimum Blue Reflectance can be used as a proxy for the reconstruction of past temperature variability, preparation and measurement standards need to be established. Sanding samples with 400 grit paper, scanning at a resolution of 1200 dpi, and image saturation of 100% were shown to produce most reliable results in the high-frequency domain. However, the results presented in this study derive from the simplest possible spruce samples evading challenges like changes in surface colour due to heartwood-sapwood transition or narrow rings of climatically sensitive trees. Therefore, further tests are necessary – including various species and large sample collections – before the settings mentioned above can be established as a standard preparation. The tests that were carried out so far suggest that many methodological challenges need to be overcome before blue reflectance can be used to reconstruct longer term climate changes, and especially those that exceed the length or age of the wood samples. In particular, the necessary calibration that "ties" measurement series to absolute density or reflectance levels remains elusive.

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