J Wood Sci (1999) 45:179–180

LETTER TO THE EDITOR

Increase of error in lignin measurement using ultraviolet microscopy due to multiple scanning

In a recent paper Okuyama et al.[1] presented a study involving lignin measurements using the photometer microscope Zeiss MPM800. Because the accuracy of measurements performed with small measuring spot diameters was of special concern, they recommended that measurements be repeated 30 times using a 1-μm spot to increase precision. Earlier researchers[2,3] pointed out that the scanning time must be kept short because photodegradation of wood sections occurs during ultraviolet (UV) microscopy. If photodegradation is substantial, repetition of measurements does not decrease, but increases, the error of the lignin measurements, as described in the following example.

A small block of *Picea abies* Karst., 0.5 mm wide in tangential and radial directions and 2 mm long, was embedded in Spurrs resin.[4] A cross section 1 μm thick was obtained on an ultramicrotome using a glass knife and subsequently mounted on a quartz slide and embedded in a glycerol-water medium. UV absorption spectra were determined from 260 to 300 nm using the microspectrophotometer Zeiss MPM800. The diameter of the measured spot was 1 μm, monochromator bandwidth was set to 5 nm, and stepwidth was 2 nm. An Ultrafluar objective (100×, N.A. 1.20) and an Ultrafluar condenser 0.8 were used. Altogether 100 measurements were performed on an identical site in the S_2 layer of an earlywood cell. Absorbance at 280 nm progressively diminished and the standard deviation increased (Fig. 1), which is consistent with previously published studies on photodegradation under the UV microscope.[2,3]

It can therefore be stated that the error of lignin measurements in wood cell walls using the Zeiss MPM800 increases with the number of repetitions. To avoid biased measurements, repetitions should be performed in areas previously not illuminated by UV light.

Acknowledgement Part of this work was funded by the Austrian National Bank (project 6019).

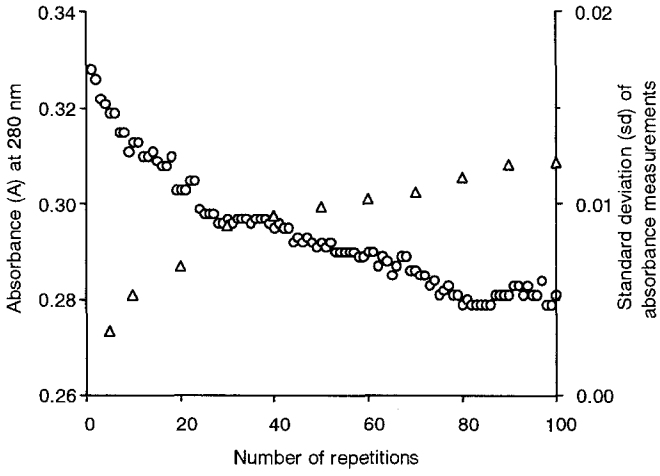

Fig. 1. Reduction of ultraviolet absorbance and increase of standard deviation with repeated scans. *Circles*, absorbance; *triangles*, SD

References

1. Okuyama T, Takeda H, Yamamoto H, Yoshida M (1998) Relation between growth stress and lignin concentration in the cell wall: ultraviolet microscopic spectral analysis. J Wood Sci 44:83–89
2. Scott JAN, Goring DAI (1970) Photolysis of wood microsections in the ultraviolet microscope. Wood Sci Technol 4:237–239
3. Takano T, Fukazawa K, Ishida S (1983) Within-a-ring variation of lignin in *Picea glehnii* by UV microscopic image analysis. Res Bull Coll Exp For Hokkaido Univ 40:709–722
4. Spurr AR (1969) A low viscosity embedding medium for electron microscopy. Ultrastruct Res 26:31–43

Wolfgang Gindl
Wood Biology and Tree Ring Research Group
Institute of Botany
University of Agricultural Sciences
Gregor Mendel Strasse 33
A-1180 Vienna, Austria

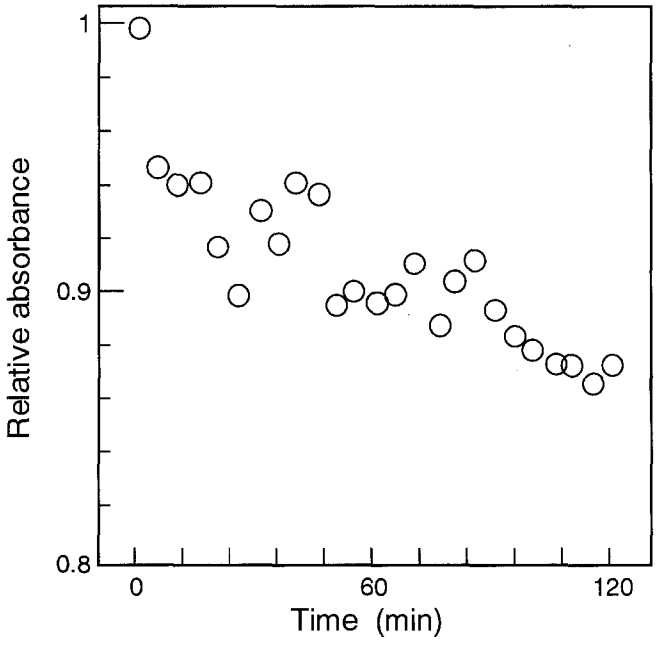

Fig. 1R. Change in the relative absorption at 280 nm ultraviolet light under continuous illumination for sugi (*Cryptomeria japonica* D. Don) of 1 μm thickness

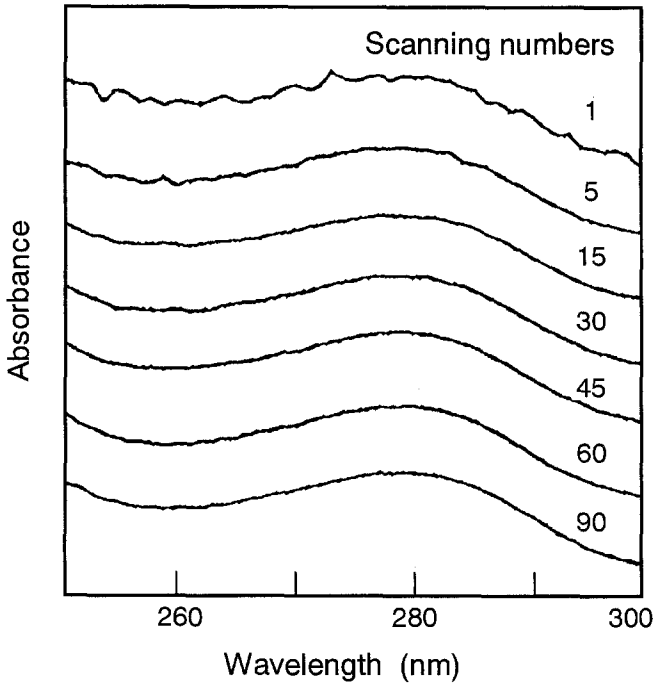

Fig. 2R. Change in the wave shape depending on the scanning repetition

Reply

Nondestructive evaluation of lignin in the cell wall is one of the most important methodologies for determining the physical properties of wood. More improvements are needed to ameliorate the qualitative analysis using UV microscopic spectral analysis.

Regarding photodegradation during UV illumination, Scott and Goring[1] reported that 75% of the UV absorption decreased during 2 h of illumination, whereas 35% reduction in the secondary wall and 20% in the middle layer of the cell corner after 2 h were reported by Takano et al.[2] Our preliminary examination of the paper showed a 12% reduction after 2 h of illumination by UV light at 280 nm wavelength (Fig. 1R). We presume the difference is due to the device used.

As pointed out by Dr. Gindl, the repetition should be performed in an area previously not illuminated with UV light, but the other error factors should also be taken into consideration. It would be the best if we could determine the precise absorption by only one scan, but we must take an averaged value of the scans to smooth the adsorption curve.

Figure 2R shows the change in the wave shapes depending on the repetition; the absorption waves of lower repetition include errors due to the lighting and the electric noise. The error was reduced by half after 30 repetitions. A compromise condition between photodegradation and the noise gave a suitable scanning number of 45 for a 0.5-μm spot and 30 for spots larger than 1 μm. As a matter of fact, it depends on the bandwidth, so now we are investigating use of a 1-μm bandwidth to evaluate data more precisely.

References

1. Scott JAN, Goring DAI (1970) Photolysis of wood microsections in the ultraviolet microscope. Wood Sci Technol 4:237–239
2. Takano T, Fukazawa K, Ishida S (1983) Within-a-ring variation of lignin in *Picea glehnii* by UV microscopic image analysis. Res Bull Coll Exp For Hokkaido Univ 40:709–722

Takashi Okuyama
School of Agricultural Sciences
Nagoya University
Chikusa
Nagoya 464-8601, Japan