# IMAGING THE MECHANICAL PROPERTIES OF WOOD CELL WALL LAYERS BY ATOMIC FORCE MODULATION MICROSCOPY

by

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SUMMARY

Atomic Force Microscopy in force modulation mode was used to study the elastic properties of the different fibre wall layers of the tension wood of holm oak and normal wood of boco. The method is based on the measurement of the resonance frequency of the microscope lever in contact with the sample. This frequency is related to the reduced Young modulus $E^* = E/(1-\nu^2)$ of the material, supposed to be isotropic. 'Elastic' images of the cell are obtained simultaneously with the topographic images, which allows the observation of the mechanical properties of the cells at a nanometric scale. Layers G, $S_1$, $S_2$ and ML can clearly be distinguished. By comparison with known materials an estimation of the absolute modulus is given in the range 5–20 GPa, but should be considered with caution, because the inherent anisotropy of the materials has not been taken into account.

*Key words:* Wood, cell wall, mechanical properties, elastic modulus, tension wood.

## INTRODUCTION

Mechanical properties of wood depend on the organisation and on the microscopic structure of its elements. Some of the macroscopic properties can be explained by the anisotropy or the porosity of the cells, but other properties such as growth stresses, shrinkage or specific modulus (i.e., Young modulus in longitudinal direction divided by specific gravity) are essentially related to the microscopic properties of the wall layers and the structure of the cells.

Using the estimated properties of wood constituents (lignin, hemicellulose, cellulose), the macroscopic elasticity of cell layers can be derived by numerical simulation (Cave 1968; Yamamoto et al. 2001), but the inhomogeneity of the wood at various scales has to be taken into account. In particular the orientation of the cellulose microfibrils in the lignin and xylan matrix influences strongly the microscopic properties of the different layers composing the wood cells (Harrington et al. 1998; Watanabe & Norimoto 2000).

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Local measurements by nanoindentation reported by Wimmer and Lucas (1997) and Wimmer et al. (1997) have shown that mechanical properties of each cell layer can be estimated, at least those with sufficient thickness to be considered as semi-infinite relative to the scale of the punch.

In this study, we use an Atomic Force Microscope (AFM) to estimate the elastic modulus of the wood at a nanometric scale, using the technique introduced by Yamanaka and Nakano (1996) and Rabe et al. (1996), called Force Modulation Microscopy or Acoustic Force Microscopy. In our experiments, the AFM cantilever is used as a reso-nator the frequency of which is a function of the interaction between the tip and the sample. This permits to map the elastic surface properties, thanks to a model based on the mechanics of contact, with the same resolution as the topography.

The technique has previously been used on different types of materials, such as al-loys, silicon devices, carbon fibres in an epoxy matrix, polymers, ADN molecules and living biological cells (Sasaki et al. 1998).

## MATERIALS AND METHODS

*Experimental set-up*

The used device is a commercial AFM (Digital Instruments Nanoscope 3100) working in contact mode, i. e., the tip is in contact with the sample (Fig. 1). The lever geometry is described by its length (L = 130 $\mu$m), its width (W = 35 $\mu$m) and its thickness (h = 1 $\mu$m), it presents a weak rigidity (k = 0.67 N/m) and a free oscillation frequency of 65 kHz. The tip length is about 15 $\mu$m and the radius at the apex is in the order of R = 30 nm for a new tip, and increases by wear up to R = 600 nm. Lever and tip are made of crystal-line silicon, coated with a thin WC layer in order to minimise its wear.

The contact force between the tip and the sample is detected with a laser beam that reflects on the lever and is received by photodiodes. During the scan of the sample, the
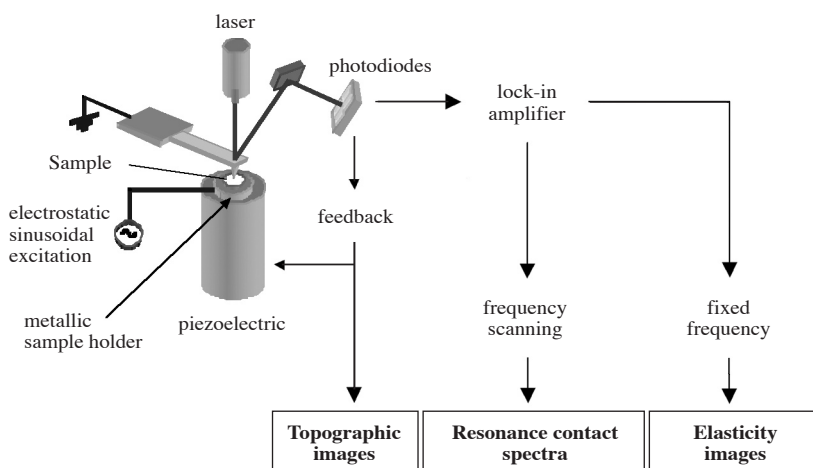


Fig. 1. Schematic representation of an atomic force microscope in force modulation mode.

feedback of the microscope maintains a constant contact force. This allows acquiring the topography of the surface, by recording the movements of the piezoelectric actuators during the scans.

Simultaneously, a periodic force is applied to the lever, by means of an electrostatic potential applied to the sample holder. When excitation frequency is close to the proper frequency of the lever a resonance occurs. The resonance frequency is related to the geometry and the stiffness of the contact, which can then be deduced.

The lever oscillation frequency (in the 500 kHz to 1 MHz range) being very superior to the force feedback response, topographic information can be separate from local variations of the mechanical property, and thus the sample stiffness and topography can be collected simultaneously.

*Quantitative analysis*

The equations governing the oscillation of the cantilever and the tip were thoroughly analysed by Rabe et al. (1998), Yamanaka and Nakano (1998), Pietrement and Troyon (2000b) and Dupas et al. (2001). For each cantilever a master curve can be built, relating the resonance frequency to the contact impedance 's' on the sample. The Hertz or DMT contact theories (Maugis 2000; Pietrement & Troyon 2000a) then permit an evaluation of the reduced elastic modulus E* of the sample, assuming an isotropic sample and a rigid tip:

$$s = \frac{dF}{d\delta} = \frac{d}{d\delta}\left[\frac{4}{3} E^* R^{1/2} \delta^{3/2}\right] = 2E^* R^{1/2} \delta^{1/2} = (6E^{*\,2}RF)^{1/3}$$

$R$ is the tip radius, $\delta$ the penetration, $F$ the contact force, and $E^*$ the reduced Young modulus (indentation modulus) $E^* = E/(1-\nu^2)$. The tip is here supposed as perfectly rigid. Note that the contact force F includes the known applied force, the adhesion plus capillary forces, and the mean electrostatic force used to excite the cantilever. The total force is estimated for each experiment (by pulling away the tip to break the contact) and ranges between 10 and 100 nN, depending on the tip apex conditions.

Many sources of uncertainty arise in the experiments. First of all the uncertainty of the cantilever characteristics as given by the constructors; for instance, dispersion of a factor 2 is common for the cantilever stiffness. Secondly, the exact shape of the tip apex may differ notably from a sphere (even for a new tip) and may evolve by wear during the scans towards a flatter surface. The effective radius of curvature of the tip goes typically from 30 nm up to 600 nm during the course of an experiment, even with a hard WC coating although the diameter of the contact zone remains below 50 nm. Obtaining an image with a constant radius is not so easy and regular tip exchange and subsequent re-calibration are needed.

In conclusion, calibration of the elastic properties of the sample cannot be done by using theoretical models only, but need to be compared to standard samples of known properties. For this purpose we have selected and measured (by acoustic methods) several homogeneous samples with properties overlapping with values expected for wood: gold (E* = 95 GPa), Pyrex glass (E* = 62 GPa), sulphur (E* = 13 GPa), PMMA (E* = 6.5 GPa) and Polyurethane (E* = 0.28 GPa) samples. For the last two materials, which

are visco-elastic polymers, the mechanical properties are measured at the working frequency, near 500 kHz.

The properties of the wood were then obtained by interpolation, with a master curve fitted on the known samples.

*Material and samples preparation*

Measurements were carried out on holm oak (*Quercus ilex* L.) wood in a zone including fibres with a gelatinous layer where both G and $S_2$ layers are thick. Other measurements have been done on normal, tension wood-free boco (*Bocoa prouasensis* Aubl.) from French Guyana. Holm oak and boco are both interesting for turnery, and have very thick fibre cell walls.

A small sample is glued on the sample holder perpendicularly to the fibres by cyanoacrylate glue (gel form). The four faces of the sample are then cut to give the shape of a cube $1 \times 1 \times 1$ mm$^3$. A final surface state is given to the superior face with a new disposable microtome blade.

The upper surface state must be as flat as possible, because too much relief would modify the contact surface between the tip and the sample and affect the elastic measurements. One can observe this effect in the G layer on the left top cell in Figure 3 were undulation of stiffness appears.

The wood samples are cut in air dry conditions and measurements were made after stabilization of the sample in ambiant air.

## RESULTS AND DISCUSSIONS

Experiments on the wood samples have been performed in three steps.

First, we choose an excitation frequency for which amplitude variations permit to generate contrast, and scanned the sample at this frequency. A picture of 'elastic contrast' was recorded, as the oscillation amplitude of the cantilever during the topographic scans. At the scan velocity, a picture was acquired in around one hour.

Secondly, a scan in frequency was performed at some fixed point on the sample, in order to measure precisely the resonance frequencies.

Thirdly, the resonance frequencies were measured on the standard samples.

For valid results, the applied force and tip shape must not have changed during the three steps, the last condition appearing to be the most difficult to achieve. Examples of curves of the amplitude versus frequency are presented in Figure 2, for a typical cell (4 points on G layer, 4 on $S_2$, 2 on $S_1$ and 2 on middle lamella).

One notices a small dispersion of the resonance frequencies within each cell wall layer and a drift toward higher values of frequency from the external layers towards the internal ones (ML towards G). This gives a qualitative information on stiffness: the G layer being the most rigid and the middle lamella ML the least. These qualitative results are in agreement with the classic description of these layers (based on microfibril angle and crystalline cellulose content).

The two pictures presented in Figure 3 are obtained at fixed frequencies and show how the contrast depends on the working frequency. At 620 kHz, the amplitude of the
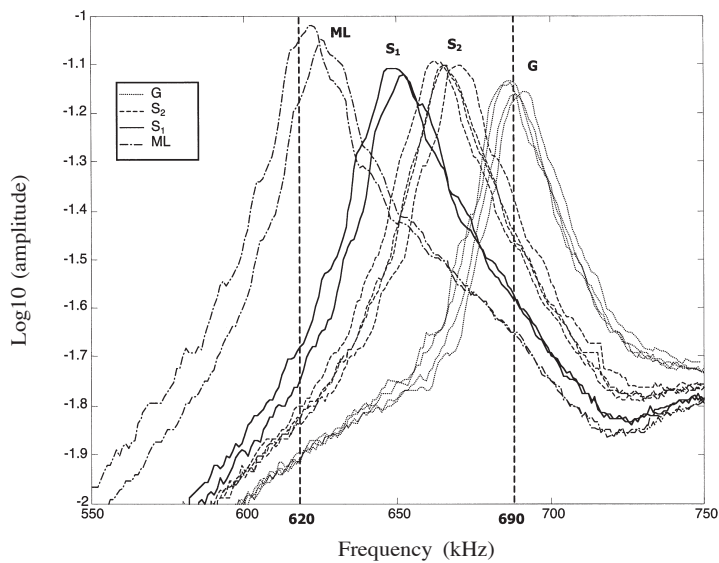
Fig. 2. Resonance spectra of the different cell wall layers of holm oak fibre.

tip is at its maximum on the soft middle lamella, and low on the stiff G layer. At 690 kHz the reverse contrast occurs.

The pictures show a large contrast between the wood cell wall layers, each layer being characterised by a relatively constant stiffness. The lateral resolution of the elastic images is in the order of 20 to 50 nm and is determined by the radius of the contact area between the tip and sample.

Quantitative results obtained by comparison with standard samples, as explained above, are reported in Table 1, together with results obtained by Wimmer and Lucas (1997) and optimal values used by Harrington et al. (1998) or Watanabe and Norimoto (2000).

Table 1. Measured elastic properties of the cell layers, in GPa, expressed as the indentation modulus $E^* = E/(1-\nu^2)$ or as the Young modulus E for extension experiments.

|  |  | ML | $S_1$ | $S_2$ | G |
|---|---|---|---|---|---|
| holm oak wood fibre* | r = 30 nm | 5–7 | 8–9 | 9–10 | 10–12 |
| other holm oak wood fibre** | r = 40 nm | 15 |  | 20 |  |
| other holm oak wood fibre*** | r = 600 nm | 7–8 |  | 9–10 |  |
| boco wood fibre** | r = 400 nm | 10 |  | 12–18 |  |
| Wimmer & Lucas (1997) on spruce wood | pyramid indentation | 5–15*** |  | 7–22 |  |
| Harrington et al. (1998) | simulation | 18 | 53 | 64 (MFA = 0°) |  |
| Watanabe & Norimoto (2000) | simulation | 33 | 54 | 64 (MFA = 0°) |  |

r = radius of curvature; * = our experimental results corresponding to the presented curves and images; ** = other experimental results; *** = measured at the cell corner.
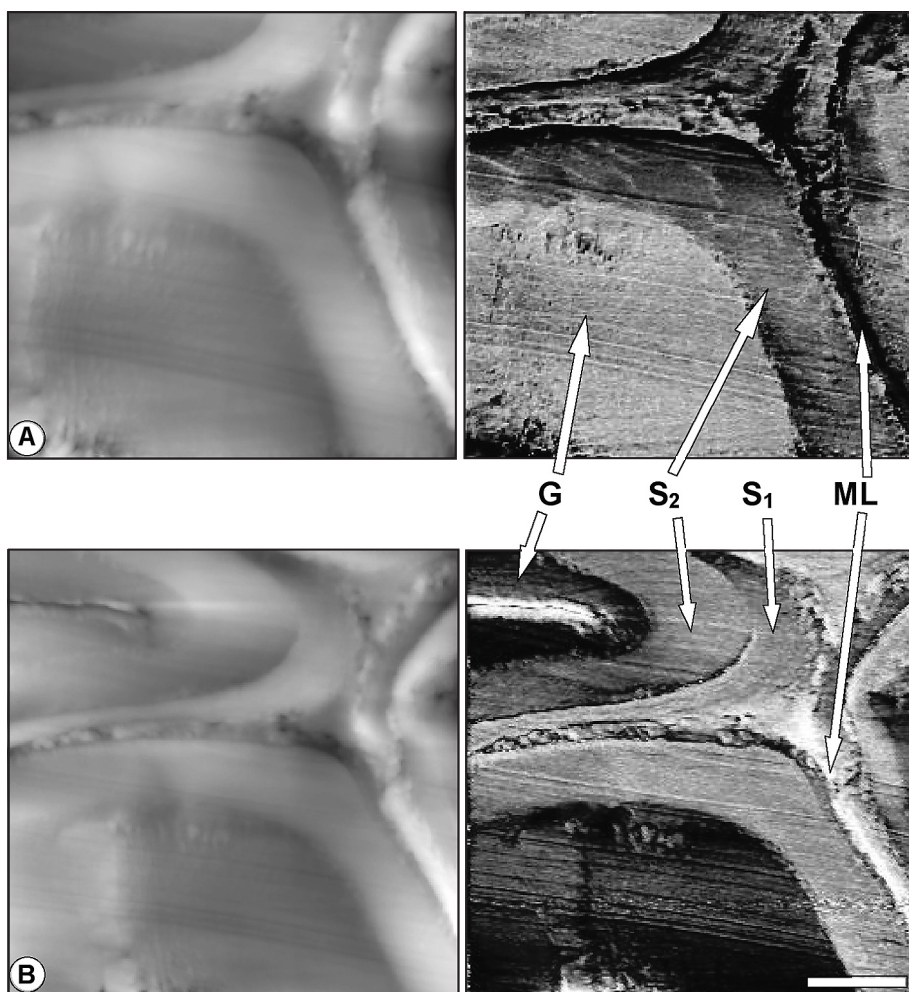
Fig. 3. Cell wall layers of oak tension wood. In topographic images (left), grey levels span a total of 300 nm. The 'elasticity' images (right) were made at 620 kHz (A) and 690 kHz (B). At 620 kHz, the lighter zone corresponds to the stiffer cell wall layers, while the reverse occurs at 690 kHz (B). — Scale bar = 2 $\mu$m.

Our results are rather similar to those obtained by nanoindentation. Wimmer et al. (1997) found large variations of the elastic modulus in each cell wall layer for the different measurements (1 to 3 in proportion). We also found such variations (1 to 2) between different measurements on the same species (holm oak). But in a same exploration on one or two adjacent cells, variations are limited to a maximum of 20% in each cell wall.

It should be noted that relative values between ML, $S_1$ and $S_2$ are similar in nearly all the cases, and that longitudinal elastic modulus estimates, both by indentation and

atomic force microscopy in force modulation methods are much lower than values used in good models (up to five times less).

On the one hand, this may partly be explained by differences in microfibril angle (MFA) of the specimens used (spruce in one case, holm oak in the other). Simulations using classical models (Watanabe & Norimoto 2000) have shown that the elastic modulus can be reduced to 20% or less when MFA increases from nearly zero to 35–40°. Normally the G layer has a very low MFA anyway.

On the other hand, the elastic modulus in both cases was calculated assuming elastic isotropic behaviour of the cell walls, which is surely not the case. Oliver and Pharr (1992), discussing the use of nanoindentation for different materials, pointed out some discrepancy between elastic modulus measured by this method and reference values for anisotropic materials (sapphire and quartz). They argued that in this case, using isotropic simplification can lead to values intermediate between the elastic moduli in the two directions of anisotropy.

In the case of highly anisotropic fibre reinforced polymers like cell walls, a ratio of ten between the elastic modulus in transverse and longitudinal direction is often found. Thus, calculated values of the elastic modulus using isotropic elastic contact (which is the case in both methods) can lead to values lying somewhere between one and one tenth of the longitudinal expected one.

Basic mechanical analyses are needed to fully understand what is really measured in both methods for cell wall material. Both measurements on transverse cell sections, as done in our studies, and on longitudinal (radial or tangential) cell sections will be necessary to estimate the elastic modulus in the two main direction of anisotropy in the cell walls at the same time.

Anyway both techniques have a high potential in the future to investigate mechanical properties at the cell wall levels for absolute values or for comparisons between cell walls or between cells representatives of different wood types (juvenile / mature; spring / summer or normal / reaction wood).

Both techniques also seem complementary. Nanoindentation uses very hard (diamond) tips of which the geometry is well know and rather stable. It has a higher potential to quickly obtain a good absolute estimate of local elastic properties on a given set of positions. Atomic force microscopy in force modulation mode uses softer (silica) tips which will be blunted rather quickly during operations. Probably using blunted tips with a 100 to 500 nm radius will be a better solution to limit variations in image acquisition. This has a higher ability to perform high resolution images of elastic constants for cell walls with a reliable relative estimation of elasticity between regions.

## CONCLUSION

Modulation force microscopy was used to study mechanical properties of the cell wall layer at a nanometric level. High resolution qualitative images are relatively easy to obtain. Nevertheless, the quantification of the mechanical behaviour is only approximate, because of the simplifying hypothesis (assuming that both bodies in contact are isotropic, homogeneous, and behave in a purely elastic manner) of which the limits are still to be analysed.

For thick wall layers such as the G or $S_2$ layers, the contact surface (radius $\sim 30$ nm) is small compared to the layer width, and the measurements are the most reliable, but for thinner layers such as the middle lamella or $S_1$ layer, the boundary effect may be important, and the interaction of the tip with adjacent layers are to be taken into account.

## REFERENCES

Cave, I.D. 1968. The anisotropic elasticity of the plant cell wall. Wood Sci. Technol. 2: 268–278.

Dupas, E., G. Gremaud, A. Kulik & J.L. Loubet. 2001. High-frequency mechanical spectroscopy with an atomic force microscope. Rev. Sci. Instrum. 72: 3891–3897.

Harrington, J.J., R. Booker & R.J. Astley. 1998. Modelling the elastic properties of softwood (Part I: The cell-wall lamellae). Holz Roh- u. Werkstoff 56: 37–41.

Maugis, D. 2000. Contact adhesion and rupture of elastic solids. Springer-Verlag, Berlin.

Oliver, W.C. & G.M. Pharr. 1992. An improved technique for determining hardness and elastic modulus using load and displacement sensing indentation experiments. J. Mater. Res. 7: 1564–1583.

Pietrement, O. & M. Troyon. 2000a. General equations describing elastic indentation depth and contact stiffness versus load. J. Colloid & Interface Sci. 226: 166–171.

Pietrement, O. & M. Troyon. 2000b. Quantitative elastic modulus measurements by magnetic force modulation microscopy. Tribology letters 9: 77–87.

Rabe, U., K. Jansen & W. Arnold. 1996. Vibration of free and surface coupled atomic force microscope: theory and experiments. Rev. Sci. Instrum. 67: 3281–3293.

Rabe, U., J. Turner & W. Arnold. 1998. Analysis of the high-frequency response of atomic force microscope cantilever. Applied Physics A66: S277–S282.

Sasaki, S., M. Morimoto, H. Haga, K. Kawabata, E. Ito, T. Ushiki, K. Abe & T. Sambongi. 1998. Elastic properties of living fibroplasts as imaged using force modulation mode in atomic force microscopy. Arch. Histol. Cytol. 61: 57–63.

Watanabe, U. & M. Norimoto. 2000. Three-dimensional analysis of elastic constants of the wood cell wall. Wood Research 87: 1–7.

Wimmer, R. & B.N. Lucas. 1997. Comparing mechanical properties of secondary wall and cell corner middle lamella in spruce wood. IAWA J. 18: 77–88.

Wimmer, R., B.N. Lucas, T. Tsui & W.C. Oliver. 1997. Longitudinal hardness and Young's modulus of spruce tracheid secondary walls using nanoindentation technique. Wood Sci. Technol. 31: 131–141.

Yamamoto, H., F. Sassus, M. Ninomiya & J. Gril. 2001. Model of anisotropic swelling and shrinking process of wood. Part 2. A simulation of shrinking wood. Wood Sci. Technol. 35: 167–181.

Yamanaka, K. & S. Nakano. 1996. Ultrasonic atomic force microscopy with overton excitation of cantilever. Jap. J. Appl. Phys. 35: 3787–3792.

Yamanaka, K. & S. Nakano. 1998. Quantitative elasticity evaluation by contact resonance in an atomic force microscope. Applied Physics A66: S313–S317.