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Towards a better understanding of wood cell wall characterisation with contact resonance atomic force microscopy

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ABSTRACT

Nowadays, the multi-scale modelling of wood has a great need for measurements of structural, chemical and mechanical properties at the lowest level. In this paper, the viscoelastic properties in the layers of a wood cell wall are investigated using the contact resonance mode of an atomic force microscope (CR-AFM). A detailed experimental protocol suitable for obtaining reproducible and quantifiable data is proposed. It is based on three main steps: sample preparation to obtain a good surface state, calibration of the contact modulus using reference samples, and image processing to produce the viscoelastic images. This protocol is applied on chestnut tension wood. The obtained topography and semi-quantitative viscoelastic maps are discussed with respect to the cell wall structure, sample preparation effects, and AFM measurement specificity compared with nanoindentation.

1. Introduction

Wood is produced in successive cylindrical layers during the radial secondary growth of trees by a very thin layer of living cells located under the bark and called cambium. It is composed of several kinds of cells, organised into a honeycomb-like structure, that have a particular role for the tree [1]. From a mechanical point of view, fibres, or tracheids, are the cells that compose the tree's skeletal structure. Fig. 1a shows the typical multi-layered structure of a normal wood fibre. Each wood cell wall layer plays a particular role in the mechanical behaviour of wood, and the macroscopic longitudinal elastic properties of normal wood originate mainly in those of its secondary cell wall layer, S_2 (Fig. 1a). All layers of the secondary wall can be seen as a unidirectional long fibre composite whose fibres are crystalline (at around 70%) cellulosic microfibrils (*a priori* as long as the cell and with some nanometres in diameter), and the matrix is made up of amorphous polymers: hemicelluloses, lignin and extractives [3]. The microfibrils are helicoidally oriented in each layer of the secondary wall [4]; for example, they are inclined to the cell axis by the so-called microfibril angle (MFA), usually devoted to the angle in the S_2 layer. Moreover, fibres act like a muscle for the tree axis [5] and their (ultra)structure can differ from that of a classical structure (*i.e.* normal wood) in the case of strong axis reorientation where reaction wood is produced [6,7]. Hardwood trees, for example, produce the so-called tension wood fibres that may have a supplementary layer, generally in addition to and within the S_2 layer, as shown in Fig. 1b, with an MFA close to 0° [6,7]. This layer is usually called the "G layer" because its matrix has a gel-like structure [6,8]. This study is part of a research project on hardwood tree biomechanics that is focused particularly on the development and evolution of the mechanical properties of the G layer.

The variability in wood cell distribution, thickness and properties makes it difficult to study the mechanics of wood at the macroscopic scale (*e.g.* tree rings). Indeed, every tree and species has its own cellular organisation and structure, both of which have a strong effect on the behaviour at the macro-scale. Thus, multi-scale modelling of wood, which is used to predict, for example, the long-term behaviour of wood in structural applications, is very much dependent on the measurement of wood properties at the lowest level [9,10].

The mechanical properties at the cell wall level can be estimated using numerical computations of the properties of its constituents or, as is more usual and traditionally done, the estimations can be carried out experimentally on chemical compounds extracted from the cell walls [3]. Other measurements consist of "classical" tensile tests at the scale of the tree rings or tissue using back-calculations [11] and/or specific strain field measurements [12,13]. See Gamstedt et al. [14] for an extensive review on the use of mixed experimental–numerical methods to characterise wood properties that are not easily available by direct

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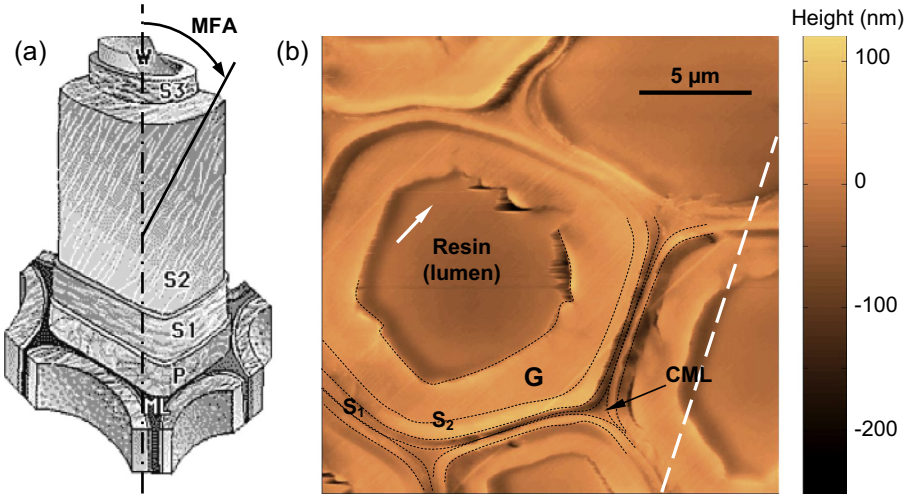

Fig. 1. (a) Idealised model of typical normal wood fibre structure, adapted from Côté [2], with the definition of the microfibril angle (MFA) from the cell axis in the S_2 layer. (b) AFM topography (256 × 256 points, 15 × 15 µm) in contact mode of the cross section of a chestnut tension wood fibre embedded in LR White resin. The cell wall consists of: ML-middle lamella that binds cells together; P-primary wall; CML-compound middle lamella that corresponds to the middle lamella ML and the primary wall P; S_1, S_2, S_3-layers of the secondary wall; G-layer only occurs for certain kinds of tension wood species; W-warty layer; Lumen-empty part inside all fibre. The arrow shows the microtome cutting direction and the dashed line the sample tilt axis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

measurements. Direct measurement at the whole cell wall scale is possible using single fibre testing and a new technique based on focused ion beam (FIB) machining of the cell wall [15]. Nanoindentation is one of the most promising and frequently used techniques nowadays. It provides access to the *in situ* mechanical properties within cell walls with as few modifications as possible; nevertheless, it does not provide access to the elastic properties but only to a complex combination of them through the so-called indentation modulus [16]. This technique has already been applied to estimate the indentation modulus of some of the wall layers of native or thermo-mechanically modified cells [15]. However, as it is commonly accepted that the radius of the elastically affected volume around the indenter is about three times the residual indent size for an isotropic material [17], this technique requires the layer thickness to be at least three times greater than the indent size; that is, typically on the order of a micrometre, to avoid measurement artefacts [18]. As the width of the cell wall layers varies from about 0.1 µm (primary wall) to less than 10 µm (S_2 and/or G layer), the interpretation of the measurements obtained by nanoindentation in the presence of a properties gradient or within a thin layer is not straightforward due to boundary effects.

Atomic force microscopy (AFM) allows not only topographic mapping at the nanometre scale but also the measurement of some of the mechanical properties (elasticity, viscosity, etc.) at this scale. Mechanical measurements by AFM, using force–distance curves, force modulation microscopy, and so on, require approaches similar to those in nanoindentation but with a spatial resolution on the order of some tens of nanometres [19]. In our case, we use contact resonance AFM (CR-AFM) mode, which is a part of the more general atomic force acoustic microscopy (AFAM) [20,21] that has already been applied on wood [22–24]. These latter studies mainly focused on the feasibility of this technique and the disclosure of the first results. While CR-AFM has now reached a certain level of maturity, the present article aims to describe a complete experimental protocol, from sample preparation to image processing. It also intends to provide a better interpretation of the images obtained on the wood cell wall. The study is restricted to the case of the chestnut tension wood cell and demonstrates local semi-quantitative measurements and qualitative mapping of the viscoelastic properties at the ultrastructural level in wood science. Furthermore, the specific structure of tension wood fibres allows us to highlight the sample preparation artefacts and to discuss them, as well as the interpretation of the CR-AFM images.

2. Materials and methods

2.1. Material and sample preparation

In order to investigate the CR-AFM imaging technique in the case of wood, chestnut (*Castanea sativa* Mill.) tension wood was selected for its ability to produce a thick G layer. This allows easier measurements in CR-AFM (*i.e.* high surface area with reduced topography) and mechanical contrast is more likely to be observed. All mechanical measurement based on indentation requires samples with a surface as flat as possible compared to the contact radius in order to be able to accurately estimate the contact area. Moreover, in the case of AFM, the tip is very brittle and surface roughness must be as low as possible to obtain reliable mechanical measurements and reduce breakage risks.

The wood samples are then embedded in a resin in order to fill the lumen and decrease the surface roughness by reducing deformation during the cutting process. Sticks (1 cm in longitudinal direction, 1 × 1 mm^2 in transverse section) are trimmed off by splitting to guarantee a good axial direction. They are then cut manually with a razor blade to produce a clear transverse surface and obtain cubes of about 1 mm^3. The samples are dehydrated with ethanol series (50%, 75%, 90% and 100%) under vacuum and embedded in an increasing ratio of LR White acrylic resin [25] using an ordinary gelatine capsule as mould. Dehydration is necessary for proper embedding and curing of the LR White resin, which is hydrophobic. Note that, in the present case, the sample could not be dried out before embedding, to avoid the dehydration with ethanol, as it would have damaged the gelatinous matrix of the G layer. Moreover, it has been shown that ethanol is a solvent with a low impact on the cell wall state [26]. LR White resin is dedicated to biological samples and is assumed to behave like epoxy embedding resins, such as Spurr or Agar, which have reduced penetration into the wall of normal undamaged wood [27,28]; to our knowledge, however, no studies have investigated the case of the G layer. Resin penetration into samples, especially into this layer, should be