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Experimental micromechanical characterization of wood cell walls

COST Action FP0802, 2008-2012: Experimental and computational micro-characterization techniques in wood mechanics

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Abstract

The properties of wood and wood based materials are strongly dependent on the properties of its fibres; i.e. the cell wall properties. The ability to characterize these in order to increase our understanding of structure-property relationships is thus highly important. This article gives a brief overview of the state of the art in experimental techniques to characterize the mechanical properties of wood at both the level of the single cell and that of the cell-wall. Challenges, opportunities, drawbacks and limitations of single fibre tensile tests and nanoindentation are discussed with respect to the wood material properties.

1. Introduction and Background

An understanding of the mechanical response of wood is essential to understand (and predict) the stability of living trees or equally important for the behaviour of wood when it is used for construction purposes. In addition to these fundamental requirements, wood micromechanical data are important input parameters in applications such as the manufacture of fibre-based products like cardboard and paper, as well as helping this resource to be used in a targeted, efficient, economic and sustainable way in for example building construction.

Wood architecture and properties

The biological material wood is formed under hydrated conditions by the living tree, mainly to ensure water transport and mechanical stability. It is a hierarchically structured material with levels (length scales) that might be termed (Speck et al. 1996) as the integral (tree as a structure), macroscopic (annual rings), microscopic (cellular), ultrastructural (cell wall) and biochemical levels (polymers). Each of these levels shows a characteristic architecture that is both dependent on the tree species and the location within the tree. In addition to this, environmental conditions are also reflected in the material architecture at particular length scales, like the formation of reaction wood, which generates active stresses that allow the tree to direct organs in particular directions (Wardrop 1965; Scurfield 1973; Archer 1986; Bonser and Ennos 1998; Yamamoto et al 2002; Moulia et al. 2006; Burgert et al. 2007; Alméras and Fournier 2009). All these dependencies of properties on wood structure are equally important both from a biological and a technological perspective. However, if we change our point of view and evaluate wood as a material for applications, the process of wood drying comes immediately into focus. Changes in moisture content lead to considerable changes of material properties at all hierarchical levels (Barkas 1939; Kollmann 1951; Niemz 1993; Eder 2007; Olsson et al. 2007). This property has led to the development of a variety of techniques (controlled drying processes, laminates) that make wood products appropriate for more and more applications. This is still an ongoing process where an improved understanding of the micromechanics of wood is highly important.

It is well known that such parameters as density, fibre orientation and moisture content, strongly influence the mechanical performance of wood at larger length scales. However, studies at the ultrastructural level, in particular on the orientation of cellulose microfibrils, revealed its considerable role on wood material properties, e.g. mechanical performance and dimensional (in)stability (Barber and Meylan 1964; Cave and Hutt 1969; Page et al. 1972; Dinwoodie 1975; Page et al. 1977; Reiterer et al. 1999) and highlighted the importance of exploring wood on small length scales. Furthermore, such approaches allow the measurement of local structural and mechanical parameters that, in turn control material performance at higher levels.

The following prominent examples should briefly illustrate research areas that might profit from experimental micromechanical characterization of wood:

- wood-fibre based industries (pulp and paper, fibreboard, wood-plastic composites)
- modelling wood mechanics – from ultra- to macro-structure
- characterization of modifications altering cell wall properties and on which hierarchical level they act
- resistance against microbial degradation (durability), dimensional stability
- biology: to understand cell wall formation and cell wall mechanics with respect to the needs of living tree – properties, structure, function

This article gives an overview of two frequently used methods that allow experimental characterization of mechanical properties at the cell (fibre) and the cell wall: single fibre microtensile tests and nanoindentation. Special emphasis will be laid on their applicability to wood. Collected data from literature are presented and will be discussed and compared with respect to the limitations of the two different methods.

2. MICROMECHANICAL TENSILE TESTS, esp. SINGLE FIBRE TESTS

A. A. Griffith, the famous English engineer and pioneer in fracture mechanics, described in his publication “The phenomena of rupture and flow in solids” (Griffith 1921) a method to measure single glass fibre strength: “...one end of a fibre was attached to a stout wire hanging on one arm of a balance, and the other end to a fixed point, the medium of attachment being sealing wax. A slight tension was applied while the wax was still soft, in order to eliminate bending of the fibre at the points of attachment. The other arm of the balance carried a beaker into which water was introduced from a pipette or burette. The weight of water necessary to break the fibre was observed and the diameter of the latter at the fracture was found by means of a high-power measuring microscope. Hence the tensile strength was obtained...” The invention of strain gages by E.E. Simmons and A.C. Ruge in 1938 facilitated force measurements considerably allowing the measurement of both smaller and larger forces. Another 20 years passed until the first tensile tests were performed on wood fibres with typical lengths ranging from 0.5 – 3 mm and diameters of 15 – 40 μm .

The first tensile tests on single wood fibres

In 1959, Jayne published his paper “Mechanical properties of wood fibres” which gives the first experimental procedure describing single wood fibre testing. He used a tensile testing machine; where forces were recorded by a load cell based on strain-gages. Strains were calculated on the basis of cross head displacement. It turned out that most of the experimental difficulties were related to the clamping of the fibres (which is still the case nowadays). Methods used in textile industry were not practicable because of the shortness of wood fibres. After several trials

with wires, glues, etc. he managed to clamp the fibres by using abrasive papers into the testing machine.

Since then ...

... many different procedures for testing have been developed see e.g. the detailed review of the early literature by Ehrnrooth and Kolseth (1984). In the following section we briefly discuss some key developments that now serve as a basis for current experimental mechanical characterization. Page and co-workers (1971) developed a frame construction where the ends of pulp fibres were glued to glass tabs. A stiff card held the glass tabs together and it was mounted on the testing machine jaws. The card was cut before the start of the test. This mounting method has been used in many investigations of single fibres, where different materials such as metal tubes or paper have been used as tabs for gripping in the tensile testing jaws. A similar principle was also applied by Burgert et al. (2002). Later, Burgert et al. (2003) enhanced the method by designing a 200 μm thick foliar frame which allowed for a certain alignment of the fibre during the test.

Another method, which allows alignment of single fibres during tensile tests, is the clamping method described by Kerseavage (1973) and Mott et al. (1995). The method is based on the application of epoxy droplets to the fibre ends. The epoxy droplets are assumed to minimise stress concentrations close to the gripping of the fibre ends. Mott et al. (1995) and Groom et al. (2002) further modified and improved Kersavage's method, by changing the mounting system. This resulted in a testing procedure where the number of tested fibres could be increased significantly. The new mounting method was more favourable because it should allow the fibres to be aligned keeping them straight during loading. Recently, Yu et al. (2011b) presented another method to grip short plant fibres with epoxy glue droplets at the fibre ends.

Strain measurements

The easiest way of determining the average longitudinal strain is to calculate it on the basis of the cross head displacement (Jayne 1959). This calculation needs to be corrected in order to take, at least, the tensile machine, frame card and the clamping (e.g. glue droplets) compliance into account. To avoid this, simultaneous longitudinal strain measurement close to the fibre by video extensometry has been developed (Burgert et al. 2003). Moreover, strain fields for the surface of the fibre can be obtained by using digital image correlation in for instance an ESEM (Mott et al. 1996; Bergander and Salmén 2000a).

Cross sectional areas and stress calculation

To obtain stress-strain curves from force – displacement data, the cross-sectional area of the fibres needs to be determined. In recent investigations, the cross-sectional area along the fibre was measured by confocal laser scanning microscopy (CLSM) (Wild et al. 1999; Groom et al. 2002; Sedighi-Gilani and Navi 2006; Hornatowska 2009; Yu et al. 2011b) or by environmental scanning electron microscopy ESEM (Burgert et al. 2003). If the fibre walls are stable enough and not collapsed, stresses can be determined both for whole cell cross

section (cell wall and lumen) and for cell wall cross sections by dividing the measured force by the measured area.

Stress-strain curves

The calculation of the stress together with the deformation allows a stress-strain curve to be plotted (a schematic of such a curve is given in Fig 1A). From such a curve, a variety of representative mechanical parameters can be derived. The tensile stiffness (otherwise known as the elastic or Young's modulus E) is determined as the slope of the lower, linear "elastic" region of the curve. This region is characteristic of a reversible response of the material, in terms of stress (or strain) to applied strains (or stresses). The yield point is the point at which the stress-strain curve becomes non-linear, and more accurately speaking occurs when deformation becomes irreversible and the material starts to flow. The shape of the stress-strain curve is strongly structure dependent. The area underneath this curve corresponds to the energy of deformation and is often used as a proxy for toughness. The maximum stress achieved before the fibre breaks is termed here the ultimate tensile stress (UTS)

Combined methods

Combining microtensile tests with other methods allow structural changes at different length scales to be related to the material response at defined stress and strain levels. Failure mechanisms of individual fibres have been observed and studied by ESEM (Mott et al. 1995; Eder et al. 2008a; Seisto et al. 1998). Polarized light microscopy allows the visualization of areas with deviant cellulose fibril alignment with respect to the main cellulose orientation allowing the response of dislocations or nodes (defects) to straining to be studied (Thygesen et al. 2007; Eder et al. 2008b). When changes in the crystal lattice of cellulose or in microfibril angles (MFA) should be studied in more detail, in-situ X-ray experiments are a powerful method (Köhler and Spatz 2002; Keckes et al. 2003; Kamiyama et al. 2005; Kolln et al. 2005; Peura et al. 2005; Peura et al. 2006; Peura et al. 2007; Zabler et al. 2010; Montero et al. 2012). Spectroscopic methods (see review Fackler and Thygesen, this issue) combined with mechanical loading allow one to follow changes at the molecular level caused by straining (e.g. Eichhorn et al. 2001; Hinterstoisser et al. 2001; Gierlinger et al. 2006).

Variations on single fibre mechanical tests

As with macroscopic mechanical testing, the loading procedure can be varied allowing for a wide range of testing conditions on the single fibre or fibre walls. For example Wild et al. (1999) constructed a tester, allowing cyclic axial and even transverse loading to be applied to individual fibres at varying humidity and temperature. Torsional properties of wood fibres and the effects of environment have been extensively studied by Kolseth (1983) and by Kolseth and Ehrnrooth (1986). The low throughput of single fibre testing can be improved by testing multiple fibres concurrently in the Single Fibre Fragmentation (SFF) test (Ljungqvist 2004) giving a breaking strain distribution from a number of fibres. Other variations of fibre testing include the determination of the bending, or

apparent modulus of elasticity by testing wood-fibre cantilevers prepared by focussed ion beam milling, tested in bending by AFM (Orso et al. 2006) as well as compression testing using micropillars to determine the compressive strength of the S_2 layer (Adusumalli et al. 2010; Zhang et al. 2010). Measurements of transverse fibre wall elastic properties have also been demonstrated using radial double wall cut strips strained in an ESEM tensile stage (Bergander and Salmén 2000a, b).

3. NANOINDENTATION

Nanoindentation (NI) otherwise known as instrumented or depth-sensing indentation (Oliver and Pharr 1992; Oliver and Pharr 2010; Fisher-Cripps 2011) is nowadays a well-established technique to characterize mechanical properties of materials at very small length scales. In comparison with tensile tests, nanoindentation on wood is a rather “young” experimental technique. For the first time it was applied by Wimmer et al. in 1997, on the cross sectional plane of fibre walls.

The measuring principle is essentially an extension of the simple hardness test that has been used in a variety of different forms for over 250 years. In hardness testing the resistance of a material against localised and permanent deformation is measured by pushing a stiff indenter with a given geometry (e.g., cone, sphere, pyramid, flat punch) into the surface with a given fixed load and then measuring the area of the resultant indent. The hardness is then defined as the ratio of a given maximum load P and the projected residual indent area A_r (e.g. Fisher-Cripps 2011). The smaller the indent area for a given load, the more resistant and thus the harder the material is. On macroscopic wood samples, hardness is usually measured on the tangential plane with a spherical or cylindrical indenter (e.g. Janka, or Brinell, and Monnin hardness test) (Doyle and Walker 1985). The Vicker’s microhardness test is a scaled down version of the hardness test and allows experiments to be performed on smaller length scales and thus lower hierarchical levels. The hardness gives information about the resistance of a material to permanent deformation and is somewhat related to the yield stress in uniaxial tensile compression testing especially for metals (Tabor 1951; 1996).

Nanoindentation follows roughly the same procedure as hardness testing, however the load and the displacement of the indenter are monitored (and controlled) with high accuracy during the experiment. This has the major advantage that, in addition to being able to get more accurate and faster hardness values, it is also possible to extract features from an indentation curve like, among other, a parameter related to the elastic properties of the material (Fisher-Cripps 2011). A typical loading-unloading curve of a nanoindentation experiment is given in Fig 1D in which the applied force P is plotted against the penetration depth h of the indenter into the sample. From this curve it is possible to compute directly the classical hardness, without measuring the residual indent area, if the indenter shape is known. The residual depth h_r is converted to the residual indent area A_r through the so-called “shape-function” (Oliver and Pharr 2010; Fisher-Cripps 2011). The most frequently used shape, is the so-called Berkovich indenter which

is a 3-sided pyramid with a face angle $\theta = 65.27^\circ$, has the following shape function:

$$A = 3\sqrt{3}h_p^2 \tan^2 \theta = 24.494h_p^2.$$

In practice the real shape of an indenter is taken into account by calibration on fused silica.

Indentation modulus

During an experiment, the material under the tip deforms both plastically and elastically during loading. Upon unloading however only the elastic part relaxes (at least at the start of the unloading curve), which allows the calculation of an effective local stiffness of the material known as the indentation or reduced modulus, E_r . This stiffness is determined from the initial (elastic) unloading slope dP/dh (Oliver and Pharr 1992) (Fig 1D):

$$E_r = \frac{1}{\beta\sqrt{A_{\max}}} \left. \frac{dP}{dh} \right|_{h_{\max}},$$

where A_{\max} is the projected contact area under load between the indenter and the material (estimated using the shape function and the contact depth area) and β a factor depending on the indenter shape, e.g. 1.23-1.24 for a Berkovich indenter (Oliver and Pharr 2004; Jakes et al. 2008; Fisher-Cripps 2011). It is possible to record this stiffness all along the loading curve using a Continuous Stiffness Measurement (CSM) less used in wood science (Tze et al. 2007).

For elastically isotropic material, the indentation modulus can be related to the sample elastic modulus E_s if the Poisson's ratio ν_s of the tested material as well as the elastic properties of the indenter material, E_i and ν_i , are known through the following relation:

$$\frac{1}{E_r} = \frac{1-\nu_i^2}{E_i} + \frac{1-\nu_s^2}{E_s}.$$

Of course wood and most other materials (including many metals, particularly at small length scale) are anisotropic. Due to the complex deformation field under the indenter tip, the indentation modulus of anisotropic materials therefore can only be viewed as being a sort-of "geometric average" of the elastic tensor (Vlassak et al. 2003; Jäger et al. 2011a). A correct estimation of all (or some) anisotropic material elastic parameters requires more information about the material eigen directions such as its microfibril angle (Gindl and Schöberl, 2004) combined with further experiments using different load, indenter shapes and orientations (Jäger et al. 2011b).

Viscoelastic measurements

As the rate of loading is controlled during nanoindentation (see e.g. Fisher-Cripps 2011) optional holding times (Fig 1D, grey circle), usually greater than 5 s for the

S₂-layer, can be introduced prior to unloading in order to minimise viscoelastic effects during unloading (Cheng and Cheng 2004). These segments can also be interpreted to measure creep behaviour. The horizontal line in the grey circle in Fig 1A indicates a creep experiment with a tensile tester. In both cases the force is held constant for a certain period and the resulting deformation is recorded (Fig. 1B). The grey, dashed circle indicates relaxation experiments where the deformation is kept constant and the decreasing force is recorded (Fig. 1C). In principle a similar procedure can be used to measure relaxation during indentation. Other less used viscoelastic measurement mode in wood science is the nano-DMA (Gershon et al. 2010).

For other mechanical properties that can be obtained by analysing the loading/unloading curve, such as strain-hardening index, fracture toughness, yield strength and residual stress under specific conditions, as well as experimental precautions or required corrections, such as indenter apex shape, device compliance, etc. see Fisher-Cripps (2011)

Going down to the nanometre scale

To study other cell wall layers than the S₂ or cell corners and to reach the ultrastructural level, it is necessary to reduce the measurement scale. Atomic Force Microscopy (AFM) allows measurements similar to nanoindentation but at a lower scale using force-displacement curves, and sometimes dedicated tips, as demonstrated on polymers (Tomasetti et al. 1998). Such a technique has been used for measuring the elastic modulus of cellulose fibrils in bending (Cheng and Wang 2008). A large number of advanced AFM techniques are currently in development for measuring mechanical properties; see e.g. Bhushan and Fuchs (2009), however only a few have yet been applied to wood cell mechanics. To our best knowledge, mainly the so-called “Resonant Contact-AFM” (RC-AFM) has been used for estimating elastic properties in the wood cell wall (Clair et al. 2003; Bytebier et al. 2009, Nair et al. 2010). Some new techniques, that operate in the intermediate scale between nanoindentation and AFM, are currently being developed and show promise for wood micromechanics like the “Scanning Microdeformation Microscope” (SMM) that is similar to the RC-AFM but at a higher scale (Le Rouzic et al. 2012) and the ultra-nano hardness tester (UNHT from CSM instruments).

4. SAMPLE PREPARATION – METHODOLOGICAL LIMITATIONS

The following section briefly discusses the respective requirements for sample preparation for single fibre tensile tests and nanoindentation.

Single fibre tests demand the isolation of tracheids, fibre tracheids or libriform fibres out of a particular wood tissue. Depending on the particular research question, chemical or mechanical isolation can be applied. Chemical isolation procedures are the method of choice when particular isolation protocols should be followed to simulate industrial processes or to remove certain cell wall components. When the fibres should be retained in their natural condition without

altering cell wall composition and architecture, a mechanical isolation technique performed with fine tweezers under a light microscope is the appropriate method (Burgert et al. 2002).

Both isolation techniques allow the fibres to be either kept wet or dried to a defined state. Humidity changes in the environment cause an immediate response in the hygroscopic cell wall, which results in many cases in a considerable twisting of the fibre (Kolseth 1983; Kolseth and Ehrnroot, 1986). This makes it tricky to mount a fibre with defined moisture content onto a testing device. In practice, never-dried, green fibres are put into glycerine to prevent them from drying. Fibres, where a drying step is not problematic, are typically dried under glass slides and afterwards glued in the dry state and subjected to the desired climate before and during the tensile test.

Until now no tensile tester has been developed which allows the accurate determination of tensile properties of fibres shorter than 0.5 – 0.7 mm. There are no such limitations with nanoindentation. However, it is commonly admitted that the radius of the elastically affected volume around the tip is about 3 times the residual indent radius for an isotropic material. Wimmer et al. (1997) found that stiffness results depend on the location of the indent in the cell wall as the thickness of the S₂ layer in wood cell walls is ~ 0.3 – 4 µm, which is of a similar scale to this volume. Thus, a correct choice of loading-unloading conditions needs to be applied. In a more recent publication, Jakes et al. (2009a) studied the effect of the location of an indent close to an edge, which can be both a free edge or a heterophase interface, and suggested the application of a factor correcting for structural compliance. The use of embedding materials rigidifies the wood sample and helps to reduce edge effects. Besides this the required flat sample surfaces are easier to prepare on embedded wood samples, either by ultramicrotoming or polishing. The interested reader is referred to the following publications that contain discussion about the penetration of embedding resin and a variety of preparation procedures (Jayme and Fengel 1962; Fengel, 1967; Wimmer and Lucas 1997; Wimmer et al. 1997; Hepworth et al. 2000; Zimmermann et al. 2006; Kamke and Lee 2007; Tze et al. 2007; Konnerth et al. 2008).

The main limiting factor in nanoindentation experiments, besides the minimum indent size, is clearly the lack of knowledge regarding the effective wood moisture content in the elastically tested volume, and it is all the more true for embedding samples. Even when resin penetration into the cell wall can be completely excluded, swelling or shrinkage due to changes in relative humidity might be restricted or increased in both directions by the embedding material that could cause stresses in the cell walls. This might lead to alterations of mechanical properties due to the development of microcracks.

5. EXAMPLES for MICROMECHANICAL PROPERTIES

5. 1. Elastic cell wall properties

Single fibre testing

In Fig 2 tensile test modulus data are plotted against the MFA that is known to be a crucial structural parameter on the cell wall level with a strong influence on the mechanical performance (Cave and Hutt, 1969; Page et al. 1977; Reiterer et al. 1999). For single fibre measurements, each data point represents a mean value of several tests, collected both from more recent literature and partly unpublished data (Mott et al. 2002; Burgert et al. 2004; Keunecke et al. 2008; Eder et al. 2009; Wang et al. 2011). Rectangles represent modulus values of chemically isolated fibres, tested under dry conditions. Triangles show modulus values of mechanically isolated fibres (unfilled triangles – wet condition, filled triangles – dry condition). These values are lower than earlier reported ones from Page et al. (1977) a fact probably related to the fact that these fibres were pulp fibres with an eventually higher cellulose content than the corresponding wood fibres here shown. Nevertheless relative comparisons show the same trend of decreasing tensile moduli with increasing microfibril angle.

The effect of moisture on mechanical properties, the difference in modulus between wet (unfilled triangles) and dry (filled triangles and rectangles) fibres being tested in tension is much larger for fibres with high cellulose microfibril angles (zoomed-in area in Fig. 2). Kersevage (1973) reported a drop in longitudinal stiffness of ~ 10% for adult single fibres (typically low microfibril angles) due to increasing moisture content. For compression wood fibres, which are known to have very high microfibril angles, a stiffness reduction up to 50% has been reported (Sedighi-Gilani and Navi 2006; previously unpublished data in Fig. 2). This fits well with theoretical calculations of the relative stiffness reduction due to moisture induced softening of hemicelluloses, based on fibre wall lamination models (Salmén 2004). Of course, the chemical composition of a compression wood fibre is different from that of an adult wood fibre but Kolseth and Ehrnrooth (1986) showed that the content of hemicelluloses and lignin in the fibres only marginally affect stiffness reduction caused by an increase in moisture content. Hence, the very low stiffness reduction with increasing moisture in fibres with low microfibril angles can be explained by the properties of cellulose microfibrils in the longitudinal direction that are almost unaffected by the water content while at high MFA the reinforcing effect is lost and fibre properties are more dependent on the matrix properties which are strongly dependent on hydration (Salmén 2004). With an increasing cellulose microfibril angle, the shear stresses in the matrix reach a maximum at an angle of 45° and therefore lower the tensile stiffness disproportionately for wet fibres with high microfibril angles compared to those with lower microfibril angles. So far no comprehensive experimental data set on tensile stiffness as a function of moisture content and microfibril angle exists. In contrast to the moisture conditions, the influence of the isolation procedure, chemically (rectangles in Fig. 2A) or mechanically (filled triangles) onto cell wall stiffness is rather small; see also (Burgert et al. 2005).

In comparison to the properties of isolated fibres the grey circles in Fig 2 show data of tensile tests performed on clear wood specimens with a cross section of ~2 x 2 mm² (Cave and Hutt 1969). Stresses were calculated on the cell wall area in a given cross section. In comparison with the single fibre data tensile moduli reach higher values. This example nicely illustrates the ingenuity of hierarchical structuring: within the tissue fibres are restricted to twist or buckle by neighbouring cells. Twisting and buckling are deformation modes when helically

wound tubes are strained in tension (Pagano et al. 1968). A wood fibre with its dominant S_2 layer can be considered being such a tube. The different behaviour due to the different testing conditions was illustrated by Salmén and de Ruvo (1985) in a model describing the deformation behaviour of a single fibre when restrained and free to twist under tension. This model describes well the differences here shown between the testing of wood (restricted twisting) and single fibres (free twisting). Additionally fibre-cross sections may vary along the fibre length and contain local anatomical “defects” (e.g. fibre ends, pits) (Neagu et al. 2006; Sedighi-Gilani et al. 2005; 2006), which lead to stress/strain concentrations. This may give rise to a smaller apparent elastic modulus if it is not taken into account; the tissue compensates to a large amount for this. Finally, damage and kinking can occur during the isolation and subsequent sample preparation process also adds to the scatter in the data.

Nanoindentation

In Fig. 3 reduced modulus values obtained by the compilation data of nanoindentation on resin embedded wood samples in dry condition are plotted against the MFA. Each data point corresponds to the mean values reported from the literature for non-modified wood samples; with reduced modulus back calculated when necessary. Filled rectangles correspond to softwood samples (Wimmer and Lucas 1997; Gindl et al. 2004; Tze et al. 2007; Konnerth et al. 2009; Brandt et al. 2010; Jäger et al. 2011b) whereas unfilled triangles correspond to hardwood sample (Wu et al. 2009; De Borst et al. 2012). In contrast to Fig. 2 where the x-axis ends at 50° , which is a natural limitation (microfibril angles higher than 50° are typically not found in wood fibres), the x-axis of the diagram in Fig. 3 is extended to 90° because here samples can be rotated or tilted which allows indenting transversely to the cellulose fibril alignment (Brandt et al. 2010; Jäger et al. 2011b). Again, absolute values of stiffness obtained by nanoindentation in the S_2 -layer are much lower than what is normally expected for the longitudinal modulus (Fig. 2). In the case of nanoindentation, this is mainly due to the complex loading under the indenter that yields a reduced modulus that depends not only on the longitudinal modulus but also on the (lower) shear and transverse moduli (Gindl and Schöberl 2004; Jäger et al. 2011a). Anisotropic models for the reduced modulus obtained by nanoindentation have been successfully applied in the case of the softwood S_2 -layer by Jäger et al. (2011a). Such models can be used for a reverse identification of some of the elastic moduli of the S_2 -wall for details see review paper by Gamstedt et al. (this issue) and Jäger et al. (2011b).

Nanoindentation experiments also demonstrate that stiffness decreases with increasing cellulose microfibril angle. However, stiffness changes due to changes in microfibril angle are lower for nanoindentation compared to tensile tests, in particular for microfibril angles below 20° and above 60° . The rather strong sensitivity of nanoindentation tests to changes in microfibril angle in the range of $20 - 60^\circ$ implies that samples need to be properly aligned with respect to the actual microfibril angle in the indentation volume (Konnerth et al. 2009, Jäger et al. 2011b). The high data scattering in Fig. 3 refers to sensitivity of nanoindentation measurements to the wall composition and particularly to the matrix composition as this figure contains data for softwood, hardwood, juvenile, normal, opposite and compression wood as well as early, transition and late wood.

This measurement technique provides thus a powerful tool to e.g. study the effect of wood modification on cell wall properties (Gindl and Gupta 2002; Zickler et al. 2006; Xing et al. 2008; Stanzl-Tschegg et al. 2009; Brandt et al. 2010; Konnerth et al. 2010; Yin et al. 2011) or the lignification of the wood cell wall during maturation (Gindl et al. 2002).

Despite targeted modifications it is well known that wood cell wall properties are strongly dependent on moisture conditions. As mentioned in the sample preparation section, humidity controlled nanoindentation experiments are difficult to perform, particularly as the moisture content of the tested layer is unknown. However, (Yu et al. 2011a) showed a reduction of ~ 17% in the reduced modulus when the macroscopic moisture content went from 3.5% to 13%.

5. 2. “Strength”

UTS – ultimate tensile stress

As mentioned in the introductory part, UTS is a measure that can be easily determined from stress-strain curves of single fibre tensile tests. One needs to be aware that an accurate determination is difficult to achieve because the cross section is not constant along the fibre length as well as that pits and other structural inhomogeneities can be found. Still a relative comparison of data is meaningful, e.g. between different fibre types (Page and El-Hosseiny 1983; Mott et al. 2002; Burgert et al. 2004), environmental conditions (Kersevage 1973; Ehrnrooth and Kolseth 1984), different fibre geometry (Eder et al. 2008a, b) isolation procedures (Burgert et al. 2005) and modifications (Goswami et al. 2008). The fact that fibres tend to break at their weakest point makes this method highly suitable to study fibre defects, e.g. naturally caused, introduced by processing or isolation procedure (Page et al. 1972; Kim et al. 1975; Page and El-Hosseiny 1976; Eder et al. 2008a, b). These different factors might also be the reason why the influence of moisture on UTS is not very clear from literature data (Kersevage 1973; Hartler et al. 1963; Kallmes and Perez 1966; Leopold and Thorpe 1968).

Hardness

Compilation of hardness data obtained by nanoindentation in the S₂-layer of different types of cells and softwoods mainly shows that there is no obvious tendency between hardness and MFA (Wimmer et al. 1997; Gindl et al. 2004; Tze et al. 2007; Konnerth et al. 2009; Yin et al. 2011). Results are highly scattered in the range 0.35-0.55 GPa. Moreover, difference in hardness values between the cell corner middle lamella and the S₂ wall is neither significant (Wimmer and Lucas 1997; Jakes et al. 2008). The hardness increases with lignin content in developing cells (Gindl et al. 2002). In hardwoods, a similar order of magnitude has been reported (Stanzl-Tschegg et al. 2009) with no significant difference in hardness between 10 hardwood species with different MFA (Wu et al. 2009). Following the idea of Gindl and Schöberl (2004) and Gindl et al. (2004), hardness seems mainly dependant on the cell-wall matrix properties. Later results on hygrothermally or chemically modified wood strengthen this assumption. Stanzl-

Tschegg et al. (2009) show that a strong thermal treatment (220°C) weakly affects the reduced modulus whereas it significantly increases hardness of a beech S₂-layer. Steam (hygrothermal) treatment leads to a significant reduction of hardness especially for temperatures higher than 140°C (Yin et al. 2011). Yu et al (2011a) found that hardness is more sensitive than the reduced modulus with changes in moisture content. All these results show that the cell wall matrix behaviour, and its modification, is prevailing on hardness. This could be explained by the complex loading under the indenter with a strong plastic deformation that probably relies on microfibrils organisation and damage (by bending, buckling, kinking, etc.) and high strain in the matrix. Brandt et al. (2010) measured hardness perpendicularly to the fibre axis, e.g. in the radial and tangential direction of the S₂-layer, and obtained values around two times smaller than in the cross-section of the fibre. One can imagine that the indenter penetration in the cell wall, and interaction with the microfibrils network, is different for a radial or tangential loading. To our best knowledge, no detailed modelling of the plastic deformation process of wood under the indenter tip has been done. In metals a significant amount of work has been done in this direction (e.g. Tabor 1996), however this relies on the detailed knowledge of the elementary processes of plastic deformation, which is missing for wood.

5. 3. Time dependent behaviour, mechanosorptive creep

Even though they are difficult to perform, creep experiments are possible both with nanoindentation (Jakes et al. 2009b; Stanzl-Tschegg et al. 2009) and with single fibre tensile tests (Ehrnrooth and Kolseth 1984). When the humidity is changed during a creep experiment on wood the creep rate is increased. The impact of this phenomenon is considerable, e.g. it is a major cause of why cardboard boxes collapse during shipping. In contrast to “pure” creep experiments, the study of mechanosorptive creep of the cell wall is currently restricted to single fibres (Navi et al. 2006; Olsson et al. 2007; Dong et al. 2010). The decisive factor is the fast response to changing humidities of a very small, unembedded sample. Interestingly it was shown that the mechanosorptive creep seems to be diminishing with increasing MFA and may be non-existing at a MFA of 45° (Dong et al. 2010).

6. Conclusion and Perspectives

The experimental techniques of single fibre tensile test and nanoindentation allow for an in depth mechanical characterization of wood cell walls. Both techniques have limitations that are strongly related to the structure and composition of the cellular wood material. The main limiting factor for single fibre tests is currently that a certain fibre length is required, which excludes short fibres (mainly hardwood and juvenile) to be tested and the fact that isolated cell walls cannot yet be tested in their transverse directions. Sample preparation with the aid of a microlaser dissecting tool combined with new gripping systems, recently developed in the robotics field (Saketi et al. 2010, Saketi and Kallio 2011) might allow tensile tests on isolated wood cell walls in “any” direction and by controlled humidity.

The requirement to embed wood samples for nanoindentation is restricted to several types of experiments. The embedding procedure might alter previously performed wood modifications, even if it seems that classical embedding resins do not penetrate significantly into the cell wall, and is mainly restricted to limited environmental conditions. Cyclic loading – unloading which allow the detection of edge effects together with very flat (cryo)ultramicrotomed surfaces might open the possibility to indent on unembedded, humidity controlled wood samples. Moreover, precise measurements, which allow the major cell wall elastic properties to be determined, require accurate knowledge of the microfibril orientation with respect to the loading direction. The understandings of hardness values, which are matrix predominant, require more research to understand the basic mechanisms of wood plasticity at the cell wall scale. Finally, nanoindentation is a relevant tool for understanding the impact of wood modification and the origin of changes in the mechanical properties at the macroscopic scale. New techniques (e.g. AFM) may allow one to go down the scale and to measure properties in other layers than the S_2 -one and/or at the ultrastructural level.

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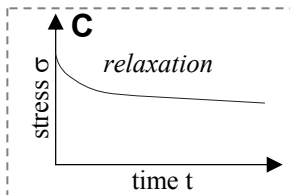
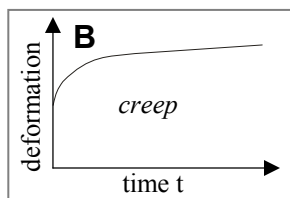
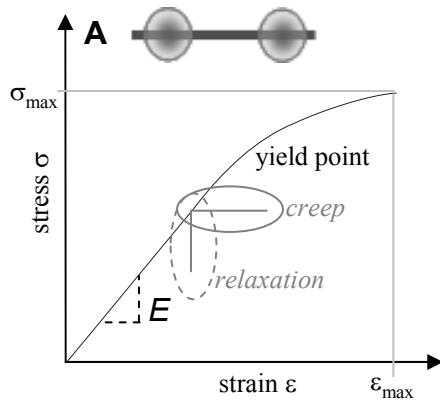
Figure Captions

Figure 1 - Schematic stress-strain and loading-unloading curves obtained during tensile tests (1A) and nanoindentation experiments (1D). 1B and 1D show characteristic creep and relaxation curves.

Figure 2 - Tensile test moduli data plotted against microfibril angles. Rectangles, filled and unfilled triangles display mean values from tensile tests on single fibres (Mott et al. 2002; Burgert et al. 2004; Keunecke et al. 2008; Eder et al. 2009; Wang et al. 2011). Grey circles show single measurements performed on 2x2mm² wood samples (Cave and Hutt 1969). Inset shows detail of the stiffnesses of high microfibril samples.

Figure 3 - Reduced moduli data (mean values) plotted against microfibril angles. Rectangles show measurements on softwood (Wimmer and Lucas 1997; Gindl et al. 2004; Tze et al. 2007; Konnerth et al. 2009; Brandt et al. 2010; Jäger et al. 2011b), triangles show measurements on hardwoods (Wu et al. 2009; De Borst et al. 2012)

TENSILE TEST



NANOINDENTATION

