"We can use wood with intelligence only if we understand wood"

Understanding Wood at the Nanoscale

– Zooming through Wood Cell Wall Ultrastructural Organization

Katia Ruel

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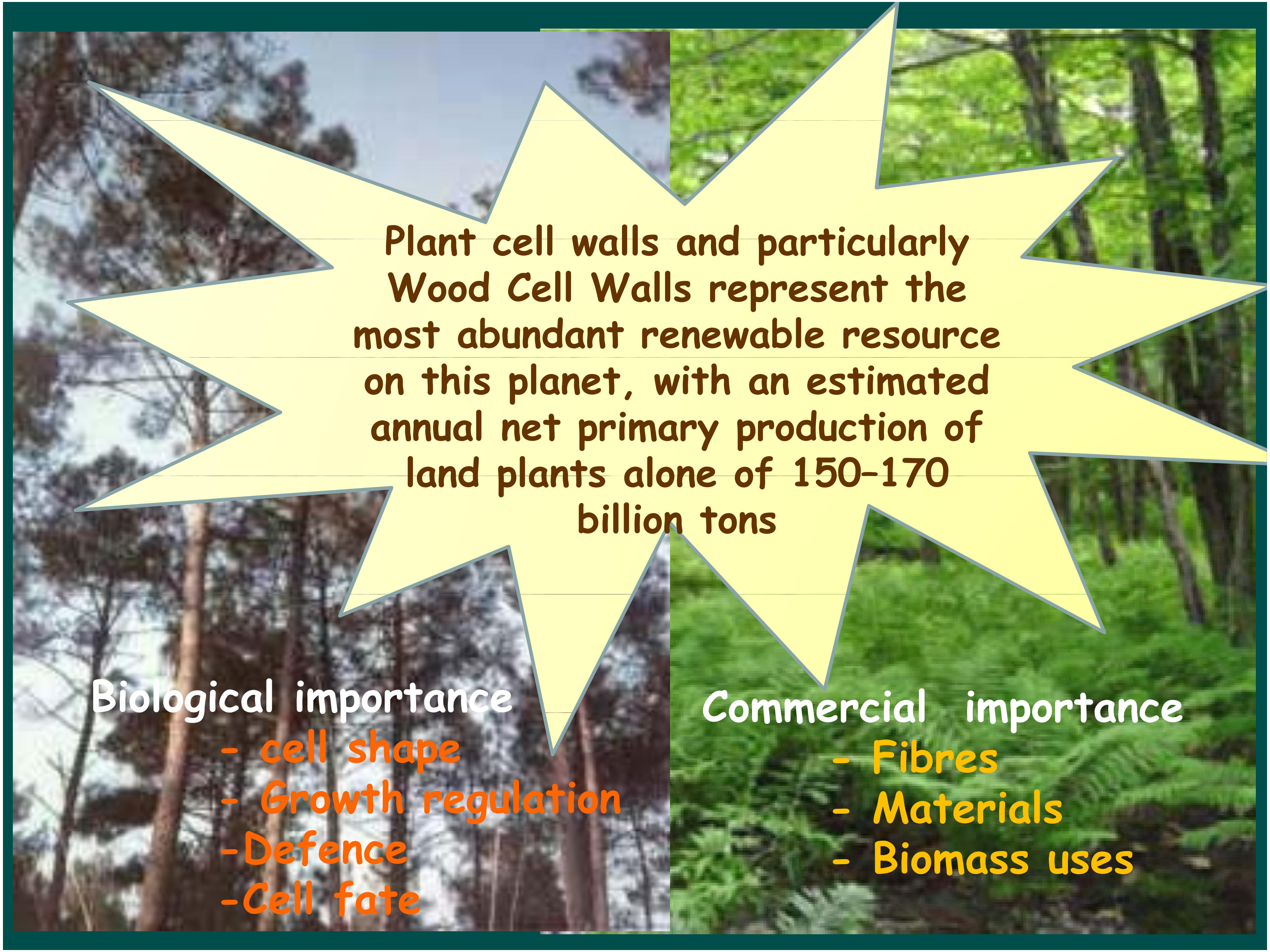
Grenoble, France

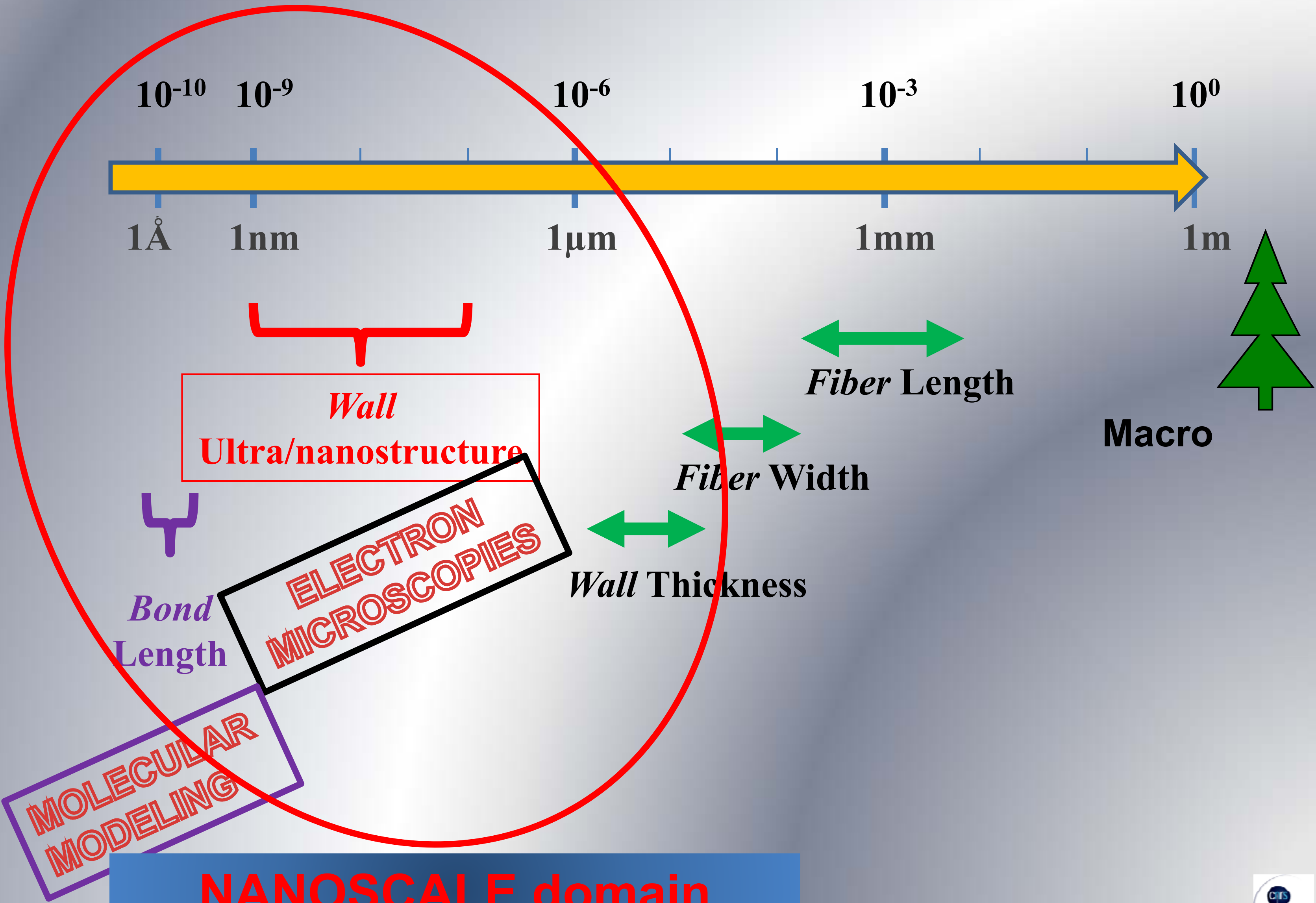
The **International Academy of Wood Science: IAWS Academy Lecture 2012**
The **Technical University in Zvolen** – ***September 26th 2012***


Plant cell walls and particularly Wood Cell Walls represent the most abundant renewable resource on this planet, with an estimated annual net primary production of land plants alone of 150–170 billion tons
Biological importance
- cell shape
- Growth regulation
-Defence
-Cell fate
Commercial importance
- Fibres
- Materials
- Biomass uses

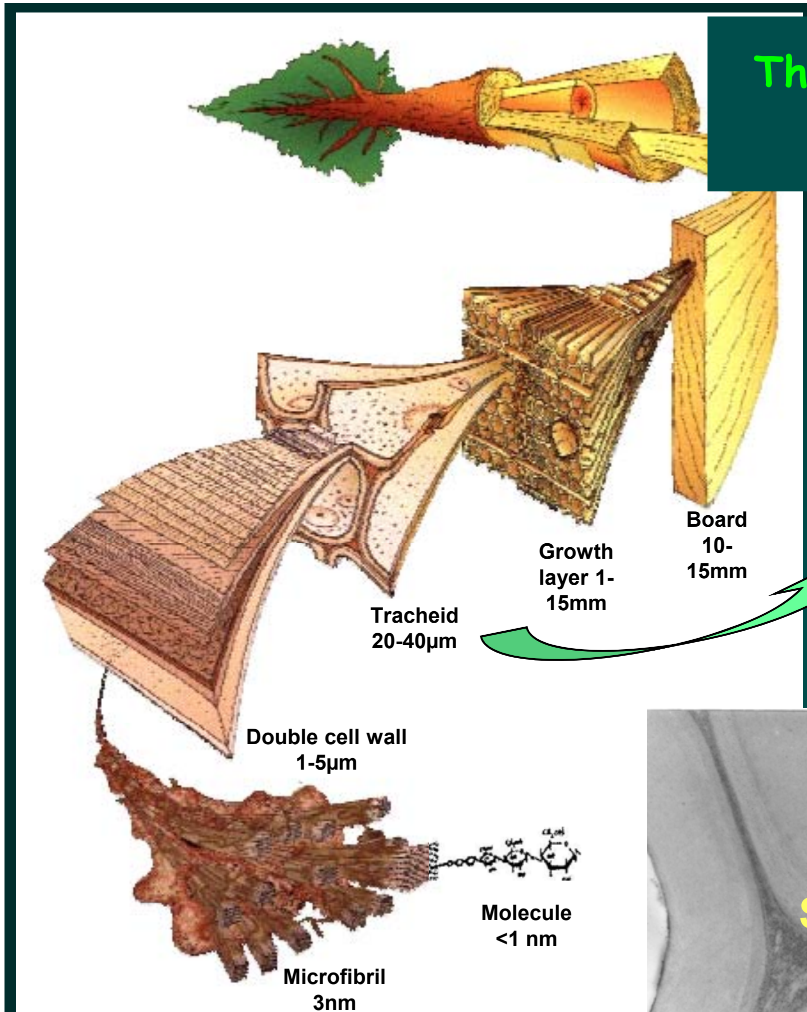
OUTLINE

- **Fundamental Elements of the Wood Cell Walls**
- **Cellulose framework**
- ***In situ* organisation of matrix Polysaccharides and Lignins**
  - Contribution of immuno-probes coupled to microscopies
  - Spatial distribution of characteristic epitopes of lignins and hemicelluloses within cell walls
- **Role of matrix components in cell wall assembly**
- **The Cellulosic Framework: *in planta* Crystalline and non-crystalline cellulose distribution**

Different scales of structural observations



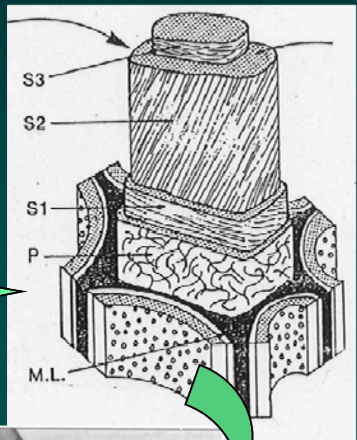
The wood cell wall is a Composite organized around Cellulose Microfibrils

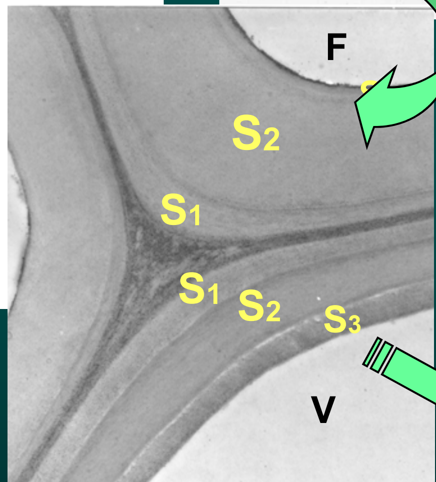


(From J. Harrington, 1998)

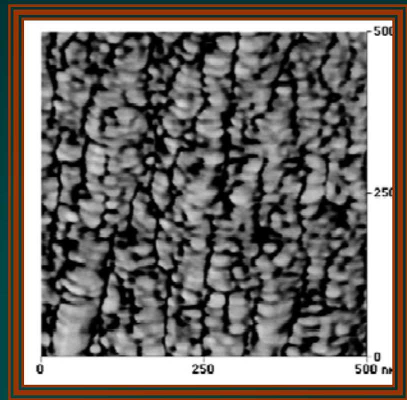
Various levels of structural Organization:

- Whole tree, Tissues
- Fibres, Tracheids, Vessels
- Cell walls: Layers, sublayers
- CMFs





the by far thickest S2 layer (more than 80% by weight of the fibre wall

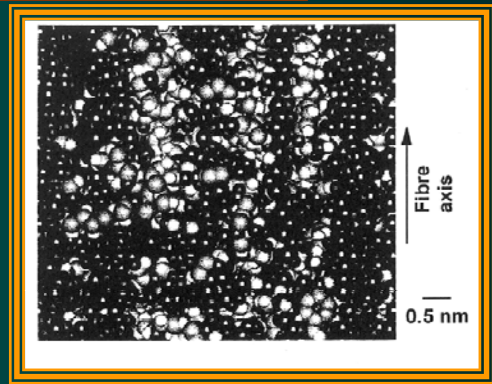


AFM

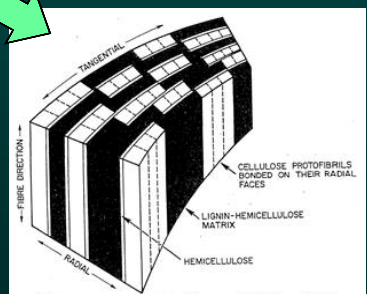
L. Salmén (2004). CR Biologies,

Molecular Modelling

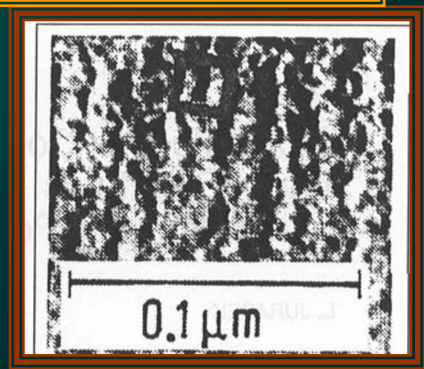
L. Jurasek (1998). In Lewis, N.G., Sarkanen, S. (Eds), ACS Symposium series, 697



TEM



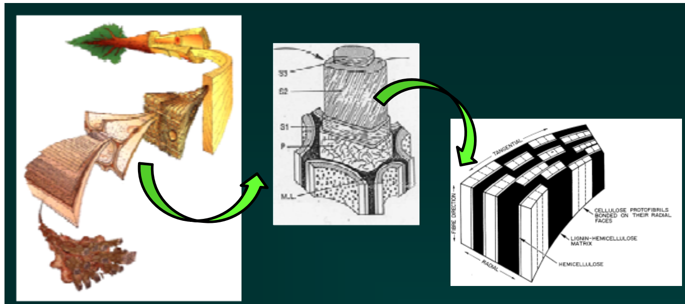
(Kerr and Goring,Cellulose Chem. Technol. 1975)



Ruel et al. (1978). Wood Sci. Technol. Concentric sublayers

◈ Fundamental Elements of the Wood Cell Wall

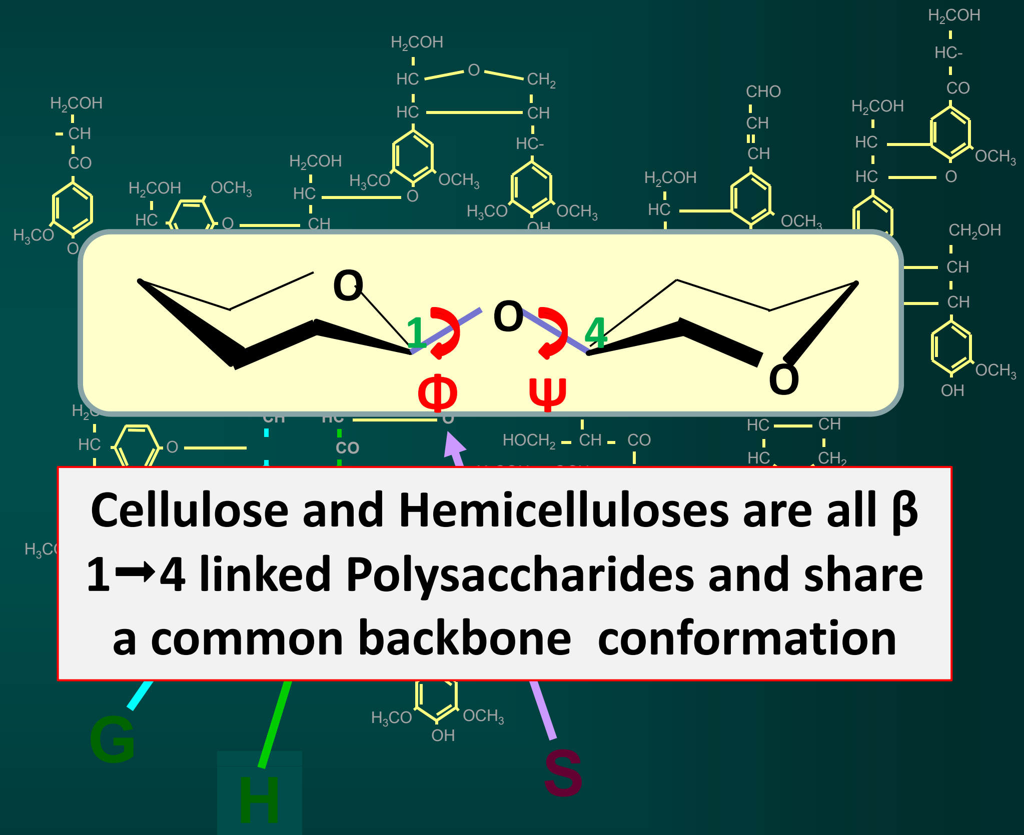
- Chemical composition



Wood cell wall components

Lignin

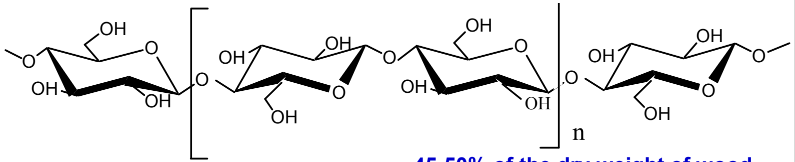
17-30% of the dry weight of wood



Cellulose and Hemicelluloses are all β 1➞4 linked Polysaccharides and share a common backbone conformation

Three basic Monomers

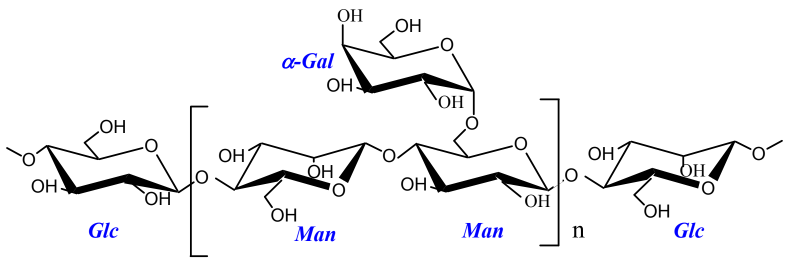
Cellulose: Homopolymer of 1,4-linked β-D-glucose; DP> 3000



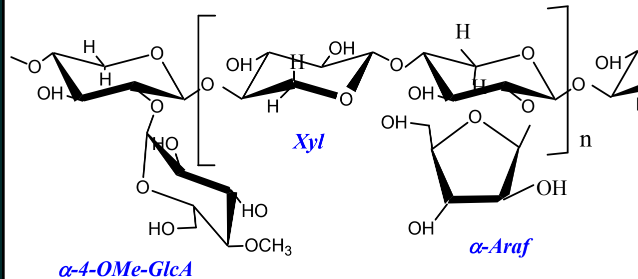
45-50% of the dry weight of wood

Glucomannan:

Polymer of alterning 1,4-linked β-D-glucose and Mannose sequences



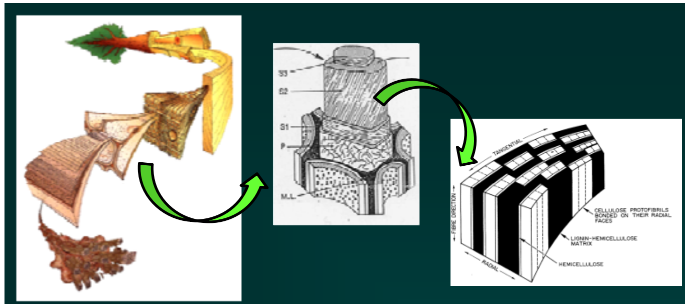
Xylans: Polymer of 1,4-linked β-D-xylose



[AX]
[GX]
[GAX]

Polysaccharides

Hemi = 25-30% of the dry weight of wood



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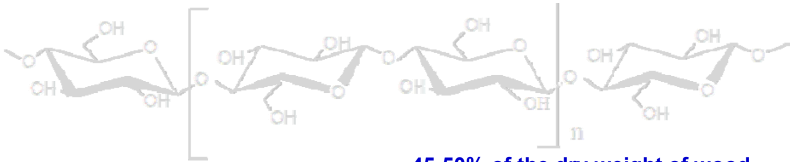

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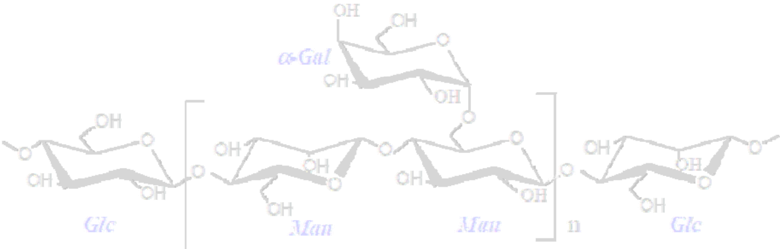

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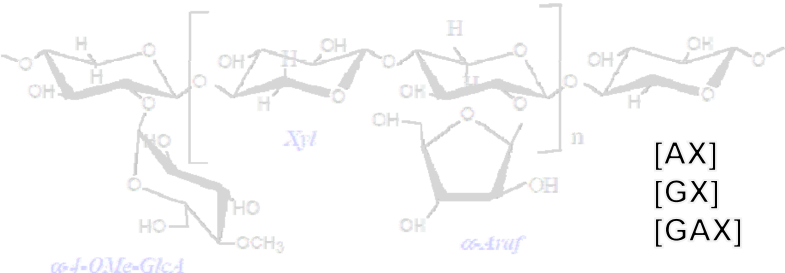

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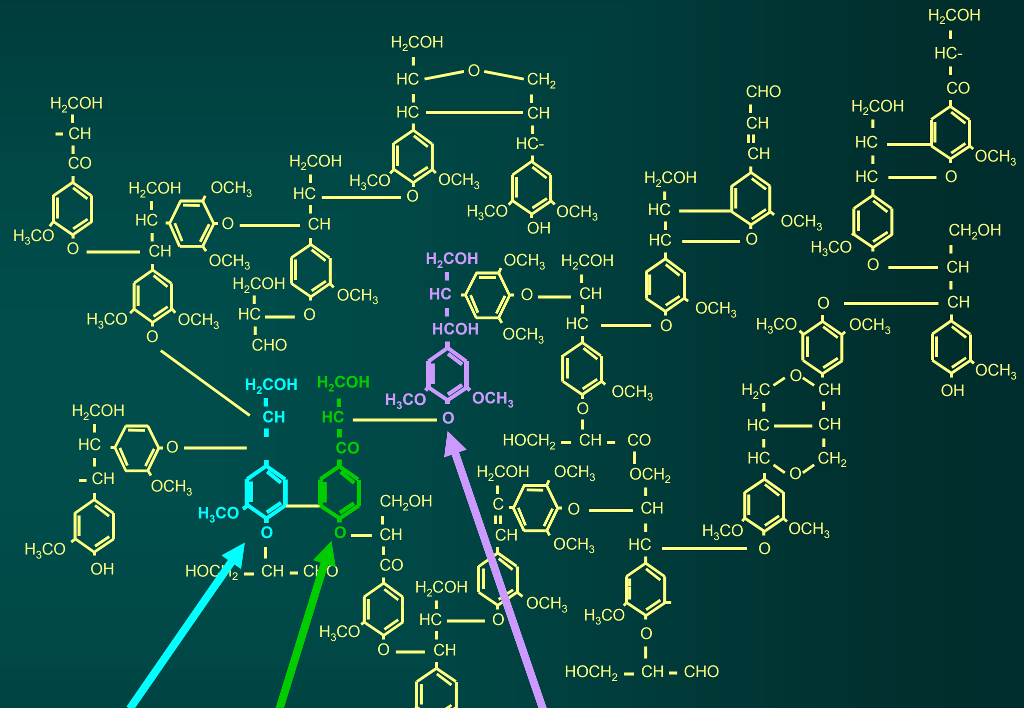
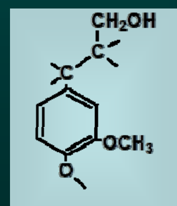
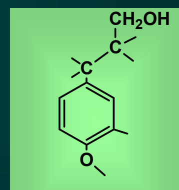


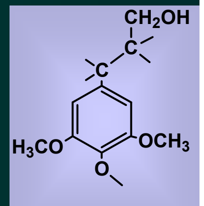
Three basic Monomers



Guaiacyl unit G



p-Hydroxyphenyl unit H



Syringyl unit S

Polysaccharides

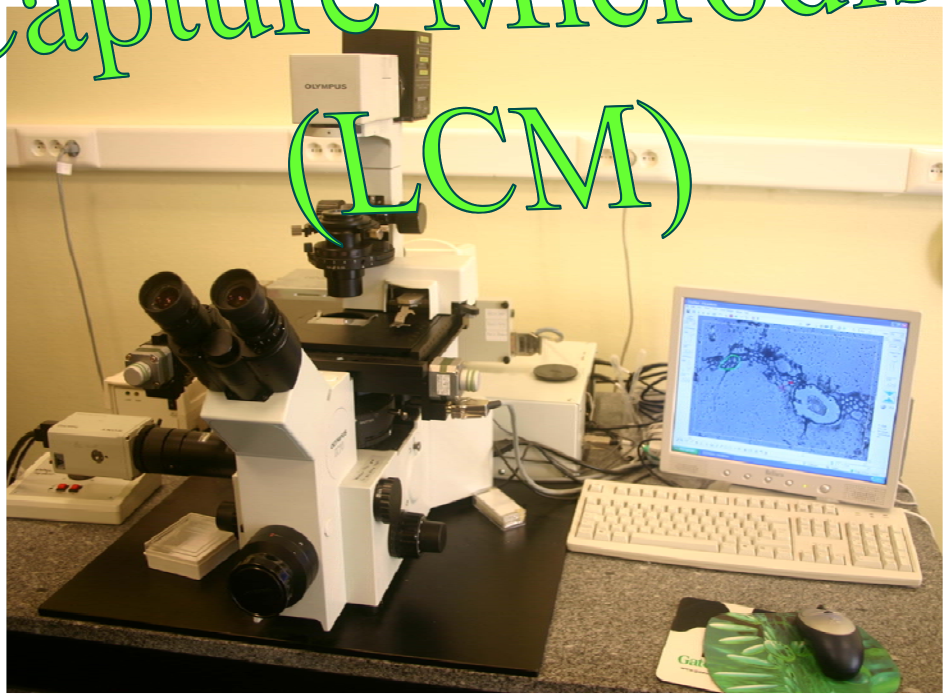
Hemi = 25-30% of the dry weight of wood

Global chemical Composition of wood cell walls: heterogeneity

	Hardwood		Softwood	
	1ry Wall	*2ry* Wall	*1ry* Wall	*2ry*Wall
Cellulose	23	**48**	21	**41**
(Arabino)GlucuronoXylan	-	**15-30**	-	**7-10**
(Galacto)GlucoMannan	3-5	**2-5**	3-5	**18-23**
XyloGlucan	20-35	**-**	-	**-**
Pectic Polysaccharides	34	**4**	32	**2**
LIGNIN (s)	-	**17- 21**	-	**24-30**

Recent method to correlating chemical analysis and tissue variability

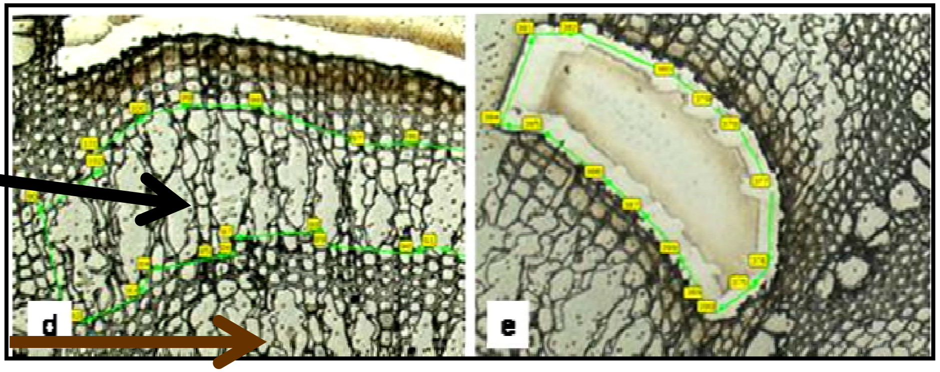
Laser Capture Microdissection (LCM)



Preparative Laser Capture Microdissection and single-pot cell wall material preparation, a novel method for tissue-specific analysis

Guillermo Angeles· Jimmy Berrio-Sierra· Jean-Paul Joseleau· Philippe Lorimier· Andrée Lefèbvre· Katia Ruel ***Planta* 224: 228-232 (2006)**

Total cell wall carbohydrate analysis from different cell types isolated by Laser Capture Microscopy technique. (LCM)



Parenchyma cells of Urtica dioica L.

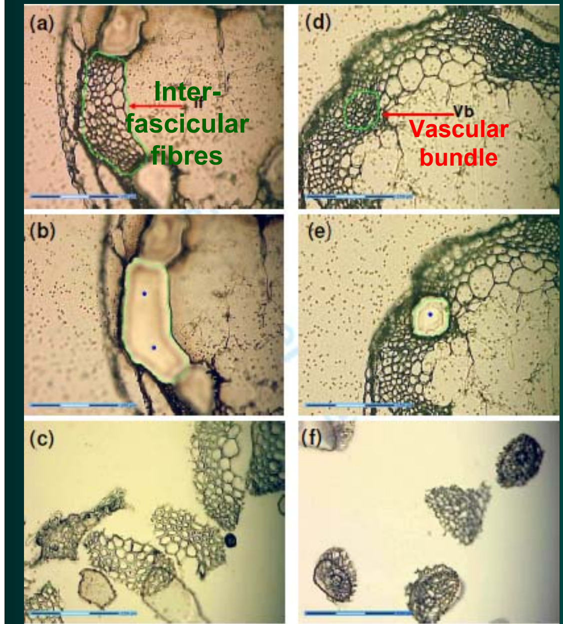
Cell Type	Ara	Xyl	Man	Gal	Glc
Unlignified Parenchyma	13 ± 3	50 ± 5	t	t	37 ± 4
Lignified Parenchyma	11 ± 3	46 ± 5	t	t	43 ± 4
Xylem Fibres	5.5 ± 1	39 ± 4	1.5 ± 1	2.7 ± 1	51 ± 4

Sugars are expressed in relative molar proportions. Values represent the average of three replicates (± SE).

(Angeles et al. Planta 2006)

Lignin analysis from different cell types isolated by Laser Capture Microscopy technique. (LCM)

Arabidopsis thaliana



if = Interfascicular fibers

vbs = vascular bundle (Vessels)

Molar ratio (S:G) and relative frequency (molar %) of the H, G and S lignin-derived monomers recovered by laser capture

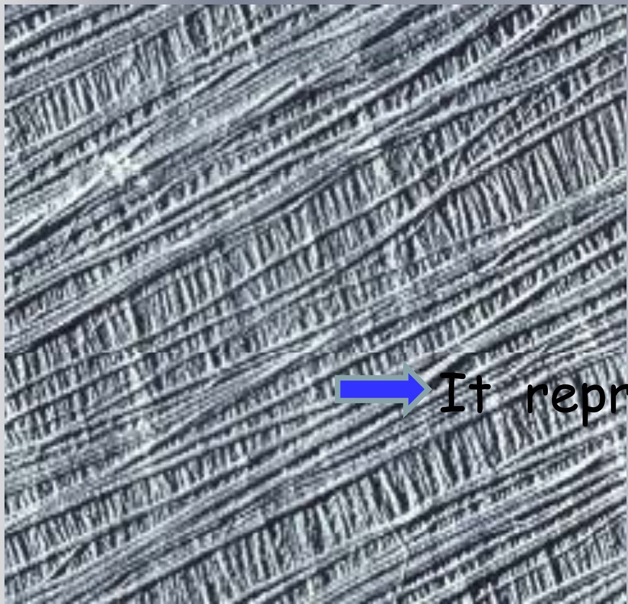
Line and tissue	S : G	H (%)	G (%)	S (%)	H + G + S
Col-0 ifs	0.64 (0.01)	0.72 (0.11)	60.7 (0.1)	38.6 (0.1)	3.02 (0.01)
Col-0 vbs	0.27 (0.01)	0.88 (0.03)	78.3 (0.2)	20.8 (0.2)	2.53 (0.05)
ccr1g ifs	0.51 (0.00)	1.6 (0.2)	64.8 (0.2)	33.6 (0.5)	1.98 (0.04)
ccr1g vbs	0.39 (0.00)	1.50	71.6 (0.3)	27.9 (0.3)	0.51 (0.03)

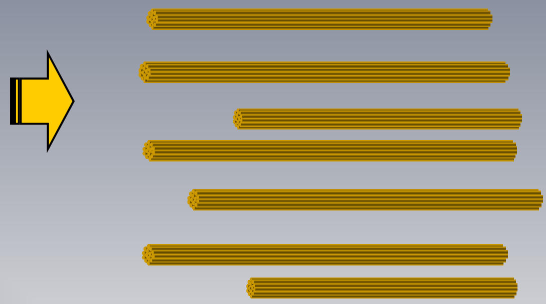
The total yield (H + G + S) is expressed in nanomoles per 100 microdissections. Standard errors between two injections are indicated in parentheses.

Tissue specific composition of fibres and vessels

Ruel et al. New Phytologist 2009

◈ The Cellulose Framework



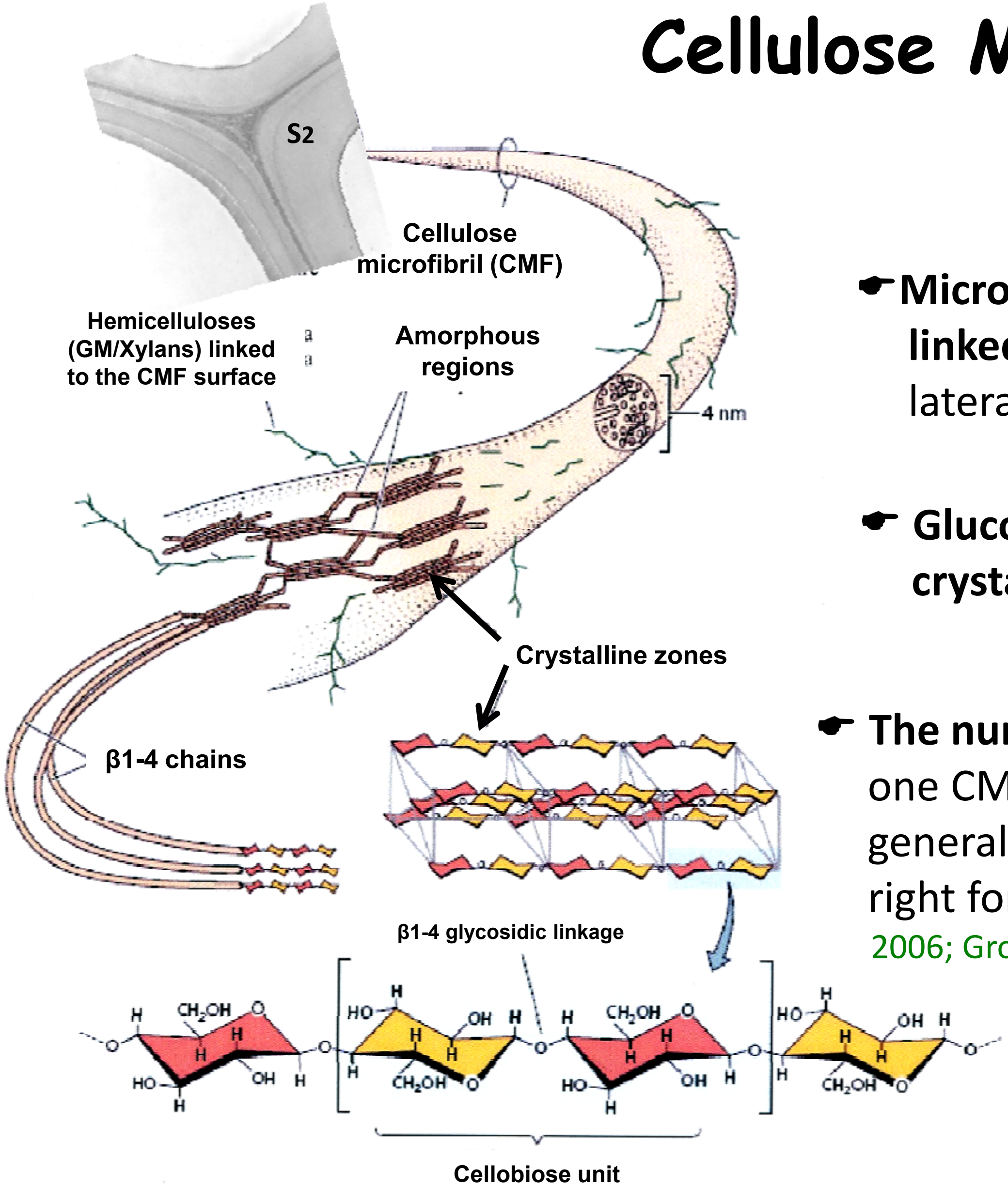


- It represents about 50% of the mass of the cell wall and forms the load-bearing structure of the plant cell wall
- May be isolated by deconstruction of the cell wall, keeping physical integrity of cellulose
- It is **the** fiber wall component of interest for new materials and polymer composites preparation

Methods allowing measurement and visualization of cellulose structure in the wood cell wall

- **XRD, XRD + Synchrotron light source** → **CMF orientation**
- **Neutron Diffraction and Deuteration**
- **Synchrotron (enhanced resolution of XRD)** → **inter and intra-molecular bonds**
- **Spectroscopic techniques: solid-state 13C cross-polarization magic-angle spinning, NMR spectroscopy** → coexistence **of two crystalline allomorphs 1α and 1β**
- **FTIR** → crystalline core surrounded by **« paracrystalline » sheath**
- **Microscopy analyses** → **cellulose dimensions**
- **Immunoaffinity probe** → cellulose orientation;visualization of the **« rosettes »**
- **AFM** → **CMF orientation, organisation, aggregates measurements**

Cellulose Microfibrils structure and organisation

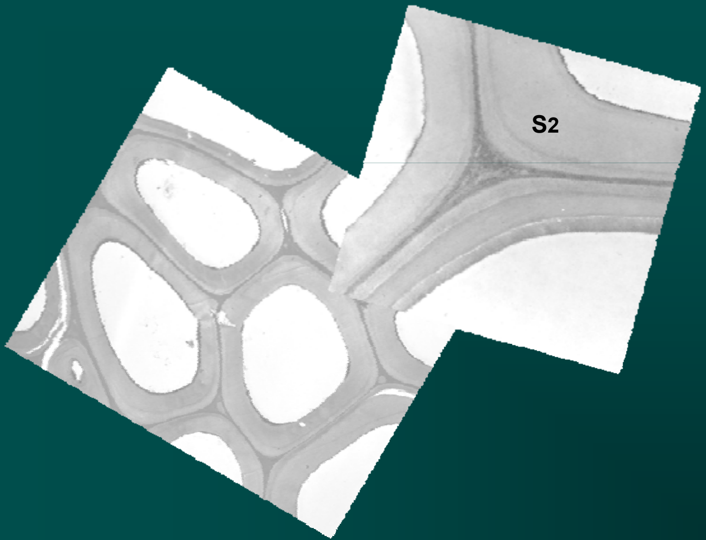


From Chanliaud (2006)

- **Microfibrils** are composed of **parallel β-1,4-linked glucan chains** that are held together laterally by **hydrogen bonds**

- **Glucose chains** are arranged in a **crystalline I** *(monoclinic)* **structure**

- **The number of cellulose chains** that builds up one CMF is **still debated**: 36 glucan chains is generally admitted but recently 24 could be right for Spruce wood CMF (Ding and Himmel 2006; Gross and Chu 2010, Fernandes et al. 2011).



PW	0.1 - 0.3 µm
ML	0.1 - 1.0 µm
S1	0.1 - 0.2 µm
S2	1.0 - 5.0 µm
S3	0.1 µm

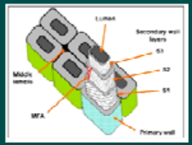
Cellulose in secondary plant cell walls

		Hardwoods	Softwoods
Fibre	Length	0.8 – 1.7 *mm*	3 – 6 *mm*
	Width	10 - 40 *µm*	30 – 40 *µm*
	Wall Thickness	3 – 8 *µm*	3 – 8 *µm*
Microfibril	Length	100 – 150 *nm*	100 – 150 *nm*
	Section	2 - 10 *nm*	2 – 10 *nm*

From Chinga-Carrasco, Nanoscale Research Letters 2011

Cellulose Microfibrils arrangement

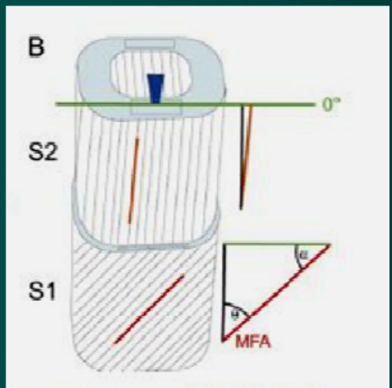
- Orientation = MFA
- Lamellar organization
- Crystallinity



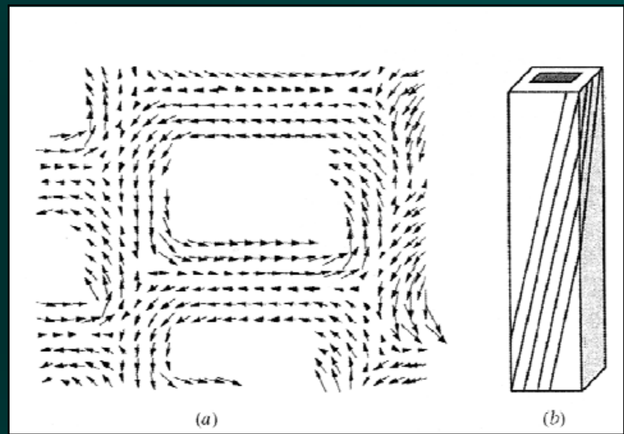
Cellulose microfibrils arrangement

Orientation of CMFs = MFA

- Importance of microfibrils orientation in fibre properties



MFA definition:
Cellulose microfibril orientation with respect to the longitudinal direction of fiber

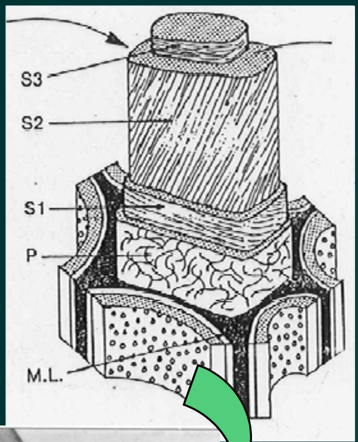


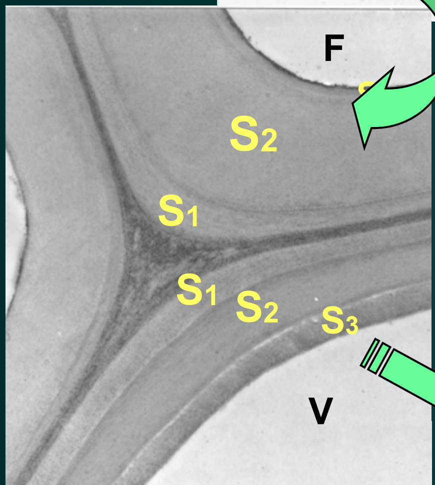
Lichtenegger et al., (1999) J.Appl.Cryst.,

Variations MFA → Consequences on properties of Fibers (Stiffness):
Large MFA = low stiffness

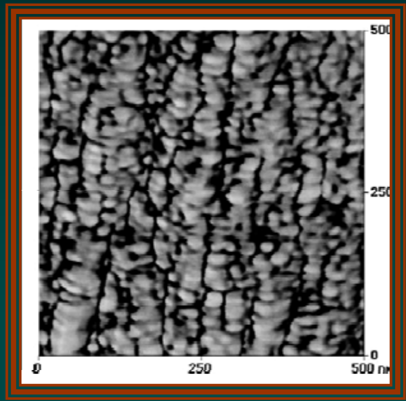
Importance for mechanical properties

The Cellulose Framework





the by far thickest S2 layer (more than 80% by weight of the fibre wall)



AFM

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Molecular Modelling

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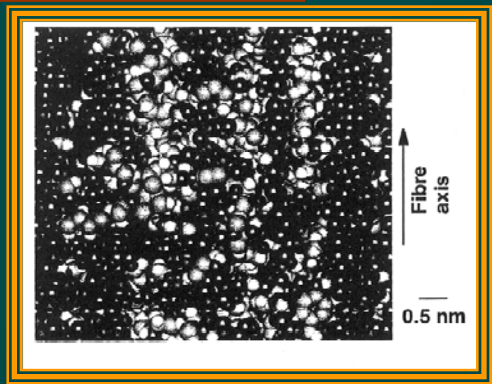
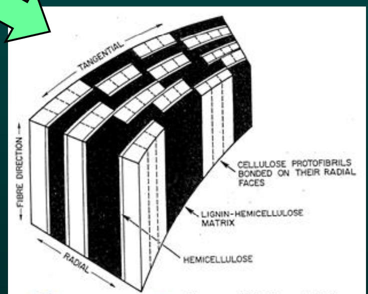
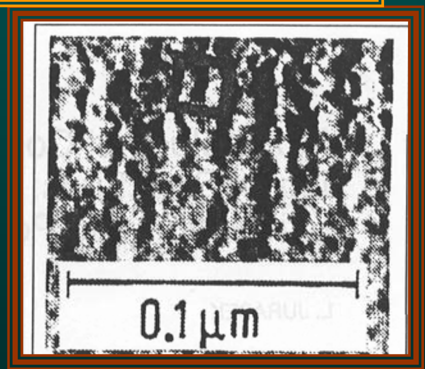


TEM



(Kerr and Goring,Cellulose Chem. Technol. 1975)



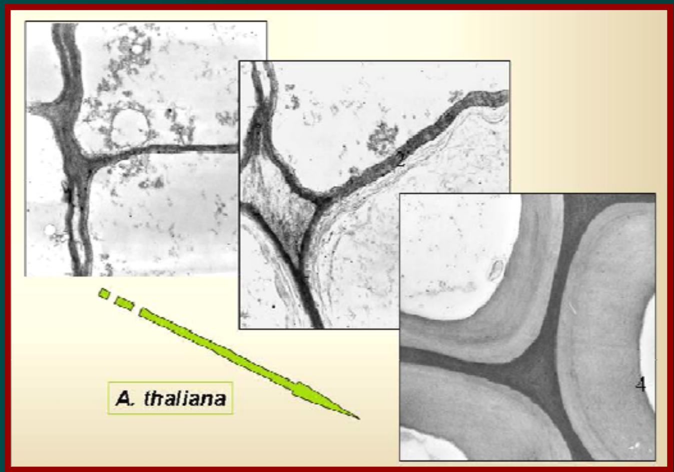
Ruel et al. (1978). Wood Sci. Technol.

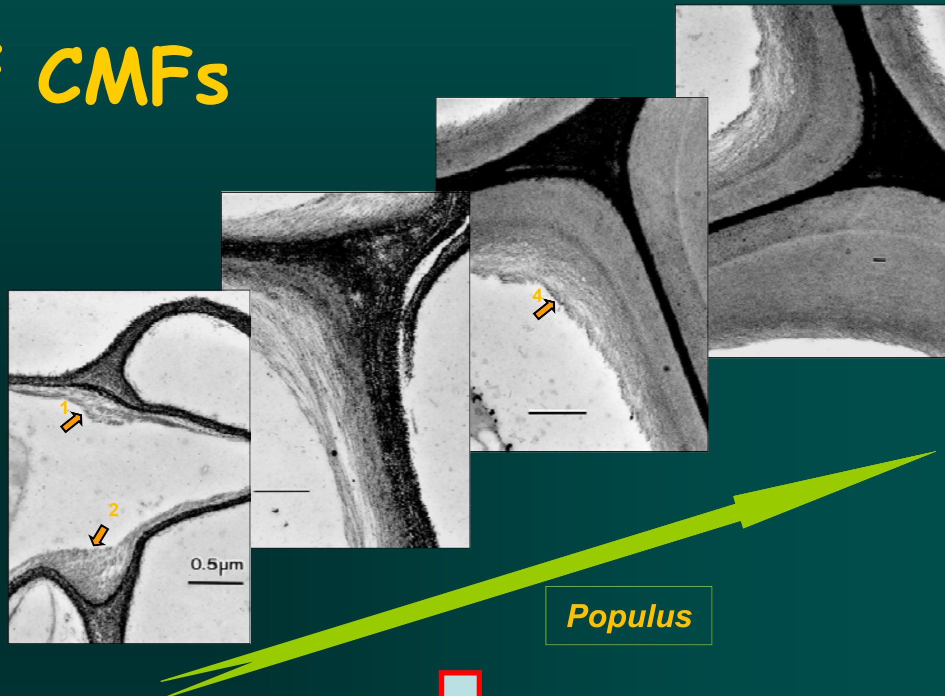
Cellulose Microfibrils can be associated to form Aggregates

TEM

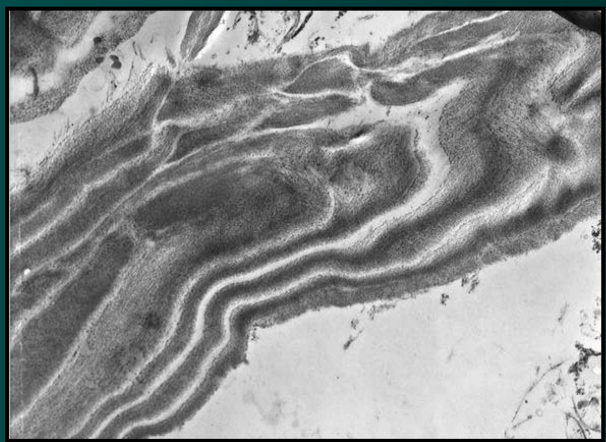
In Planta Cellulose microfibrils arrangement

Lamellar organization of CMFs





Secondary walls are the result of the apposition of bundles of cellulose microfibrils glued by matrix components

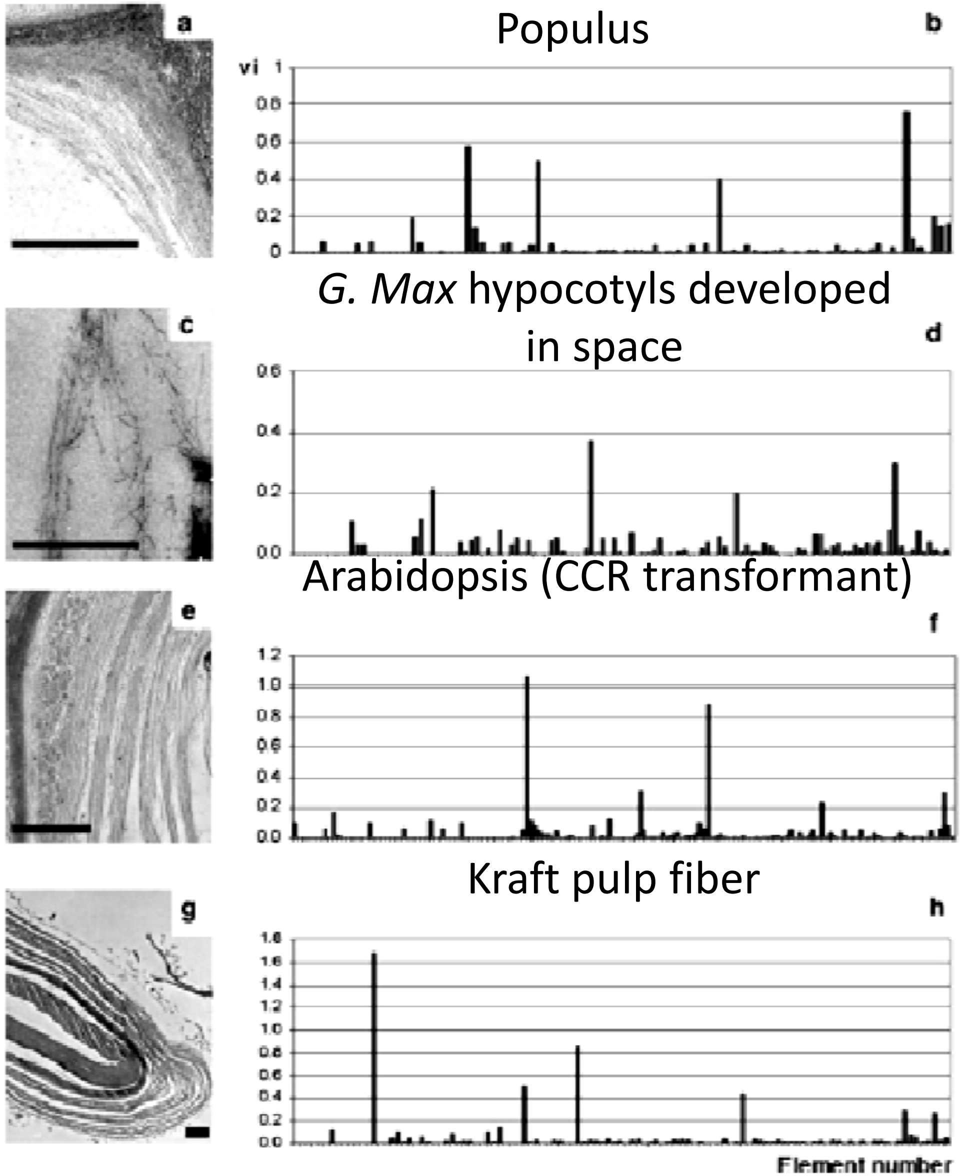


Kraft pulp

Cellulosic microfibril bundles forming lamellar substructures are basic structures of secondary walls

Katia Ruel

Common patterns of lamellar assembly of cellulose microfibrils and cell wall delamination

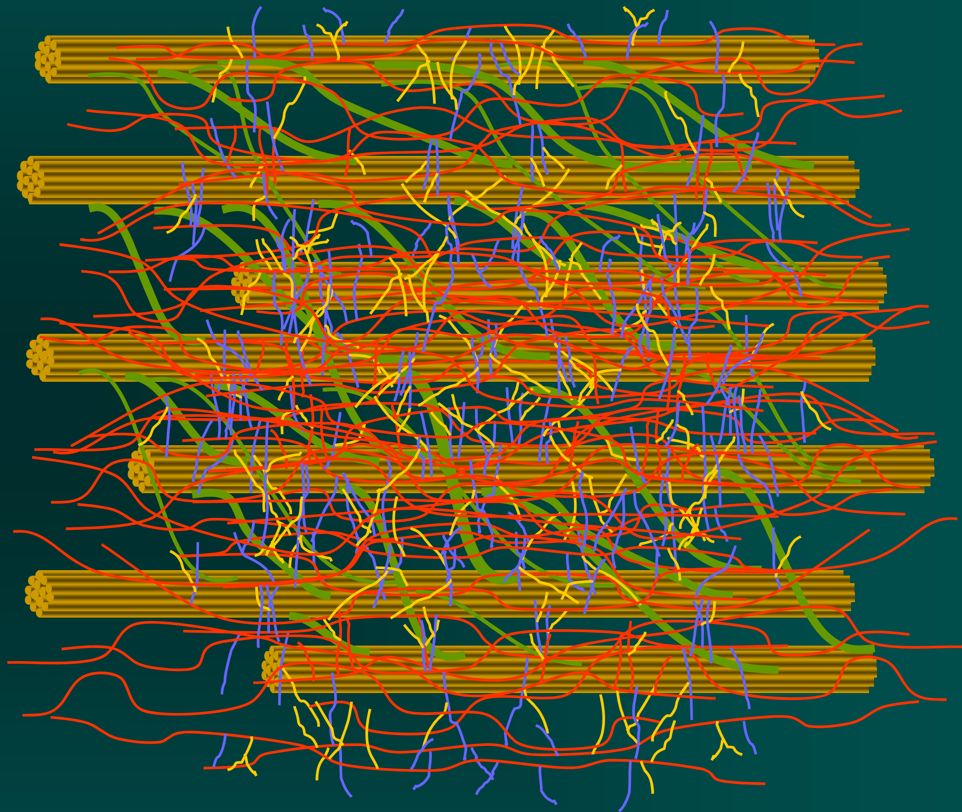


By computerised image analysis combined with graphical and statistical methods

The comparison of the patterns of microfibril aggregation/delamination in cell walls from the different origins confirmed the occurrence of a **common numerical rule, based on doubling/halving structures, during cell wall formation/degradation**.

De Micco V., Ruel K., Joseleau J-P., Aronne G. (Planta, 2010)

Fig. 1 Microphotographs (TEM) and incremental thickness (*vi*) of microfibrils and lamellae in cell walls from the four cell types: stem cells of *Populus* growing under normal conditions (**a**, **b**), cells of *G. max* hypocotyls developed in space (**c**, **d**), growing cells of CCR transformant *Arabidopsis* (**e**, **f**), pulping fibres (**g**, **h**). *Bars* 1 μm



(Adapted from S. Perez)

How do Matrix Elements fit in the Cellulose Network *in planta* ?

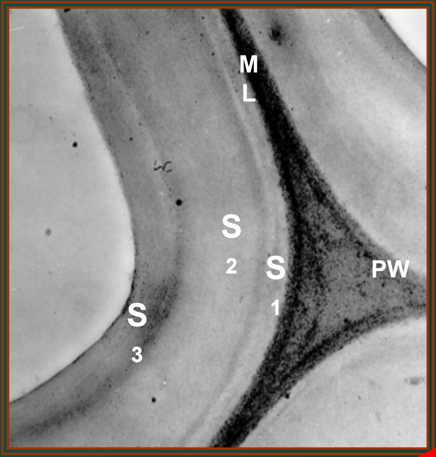
◈ In situ Organization of Matrix Components

- Hemicelluloses
  - Lignins

In situ organization of matrix componenets

☛Non-invasive methods

In planta Strategies to map cell-wall constituents



- Light microscopy (Laser confocal…)
- Micro-autoradiography
- Spectrotyping (XRD, FTIR, NIR, UV…)
- Raman spectroscopy
- Near-field microscopies (AFM,…)
- **Electron microscopy** (SEM, TEM)
- Modeling

micro/nanoscale

In situ organization of matrix componenets

Internal vibration in a molecule

FT- IR → ***Absorption*** **of radiation**

FT- Raman → ***Scatterred*** **radiation**

Fourier-Transform Infrared MicroSpectroscopy

- ► *Coupled to Microscopy* ⇨ wall polymers distribution
- ► *Coupled to Polarized light* ⇨ wall polymer orientation

[investigation under mechanical stress]

Wilson et al. 2000, *Plant Physiol*

- ► *Coupled to Dynamic Mechanical Analysis* ⇨ relationship between wall polymers and mechanical properties **Ackerholm and Salmen 2003**

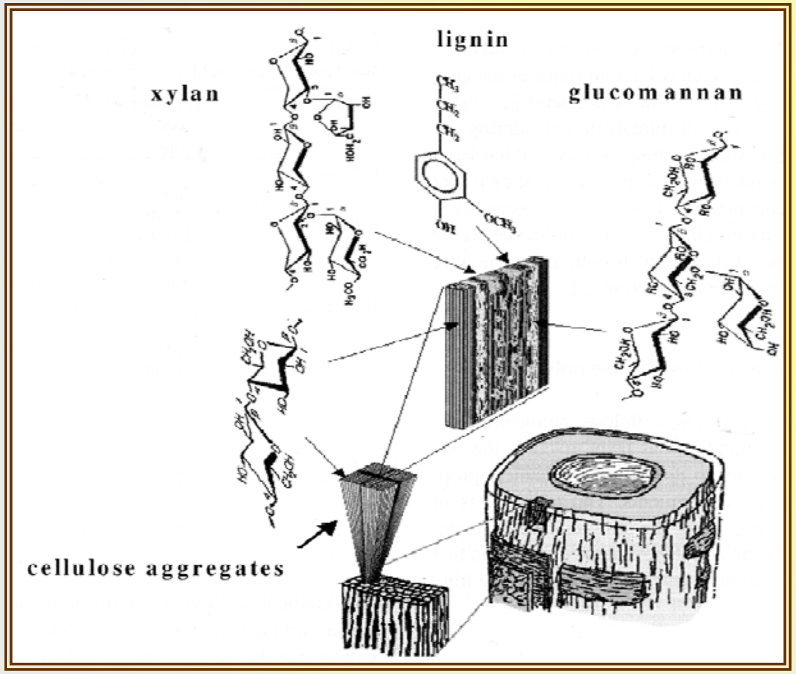
Fourier-Transform Raman MicroSpectroscopy

Gierlinger 2006, Richter et al. 2011, Hanninen et al. 2011, Perera et al; 2012.

⇨ Complementary information concerning orientation and identification of wood polymers (particularly lignin – less precise for hemicelluloses) with a spatial resolution between 0.5 and 0.1 µm

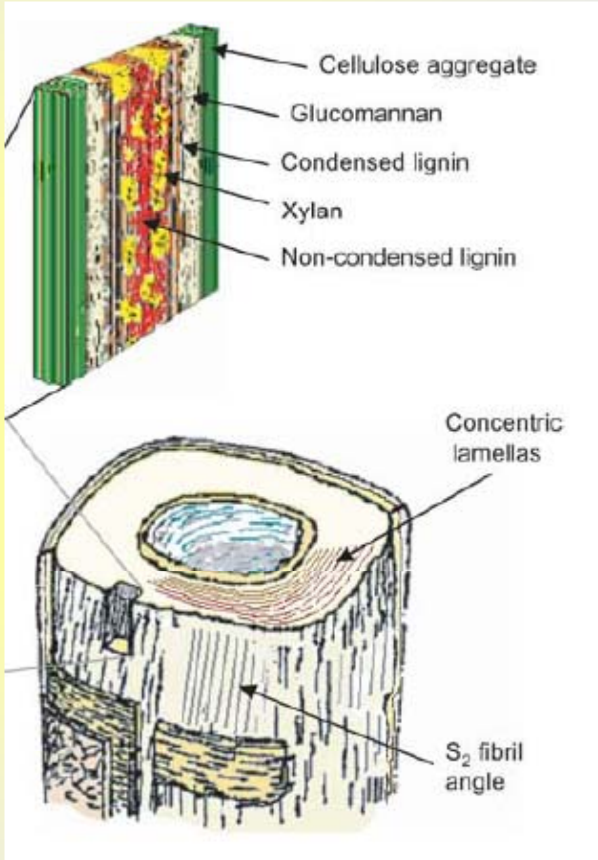
Predictive computerized model of the xylan and glucomannan interaction with cellulose

based on spectroscopic , AFM and FTIR data



From Salmen,CR.Biologies 327 (2004)

Improved with TEM data



From Salmen and Burgert Holzforschung (2009)

☛ Modelling provides indications

- about polymer most favoured conformations
- about their most favoured interactions

BUT

Not a proof of structure

Needs for better data *in planta*

In situ localization of matrix components

Non-invasive methods

immunological probes coupled to microscopies

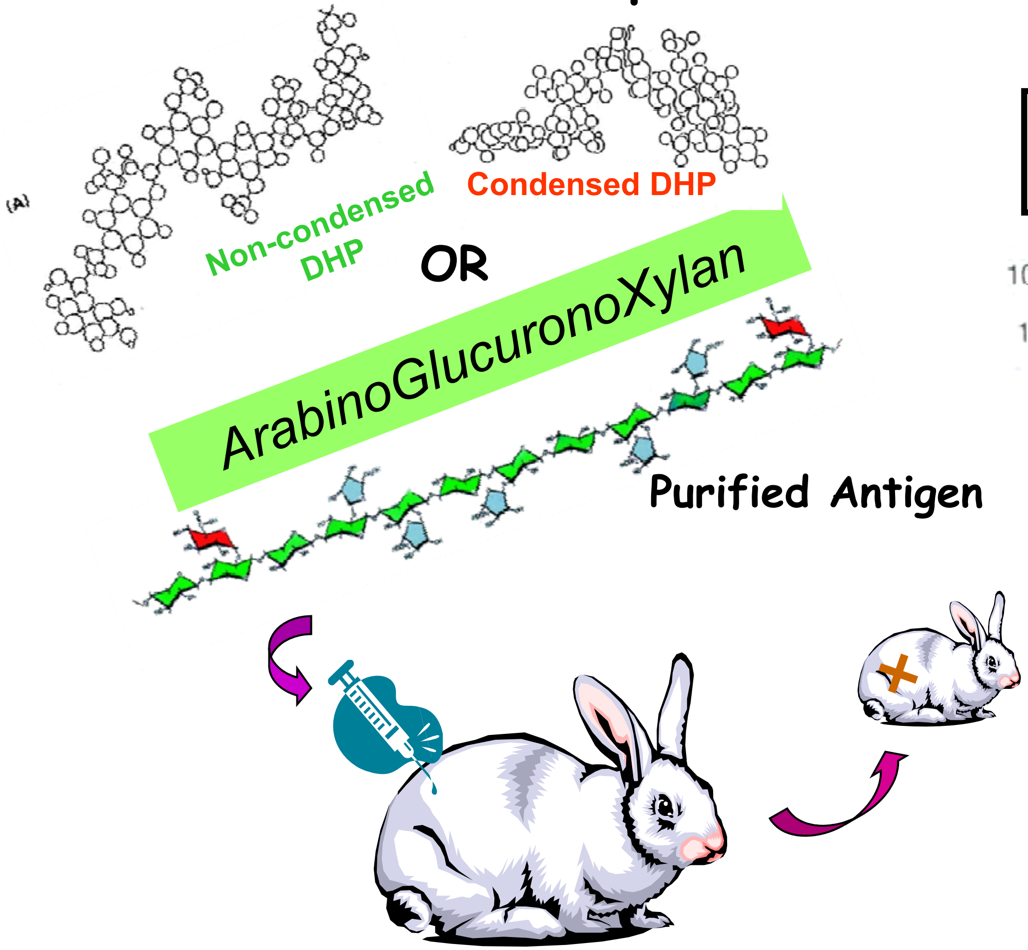
- Qualitative *Visualization* and *Identification* of the Constituents
- Respective Quantitative *Repartition*
- Spatio-temporal *Distribution*

Our Strategy for better insight *in planta*

- Prepare New molecular probes **(antibodies, Cellulose Binding Modules)**
- Genetically modified model plants
- Examination of cell wall assembly during cell growth and differentiation
- Behaviour of wall components during industrial processes **(Physicochemical, chemical, enzymatic treatments,…)**

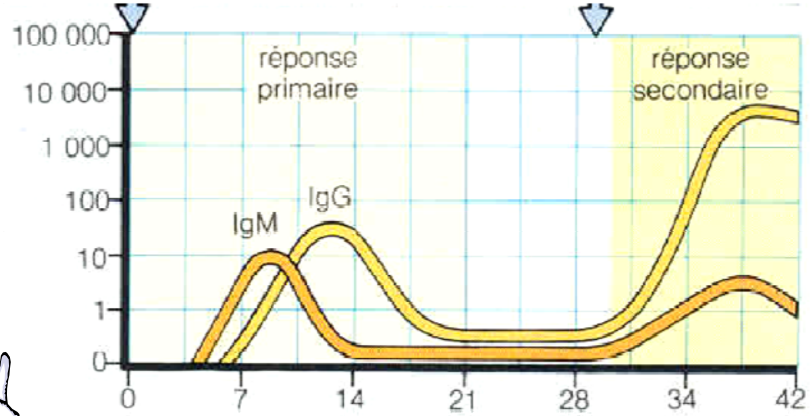
Principle of immunolabeling

Preparation of immuno-probes

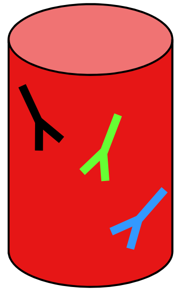


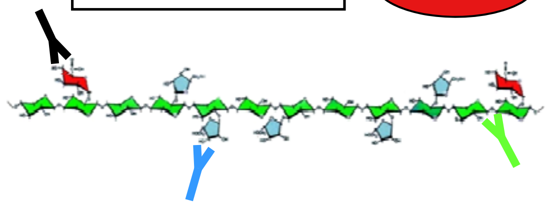
Stimulation of primary Antibodies

Stimulation of Secondary Antibodies



Polyclonal antiserum =Multiple IgGs





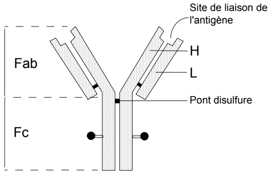
Several *epitopes* recognised

IMMUNOLABELING

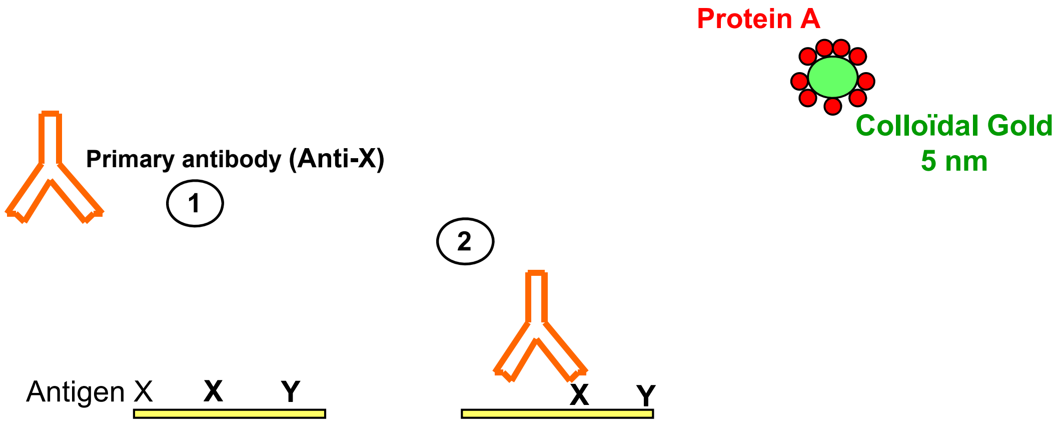
-Versatile Identification of Multiple epitopes

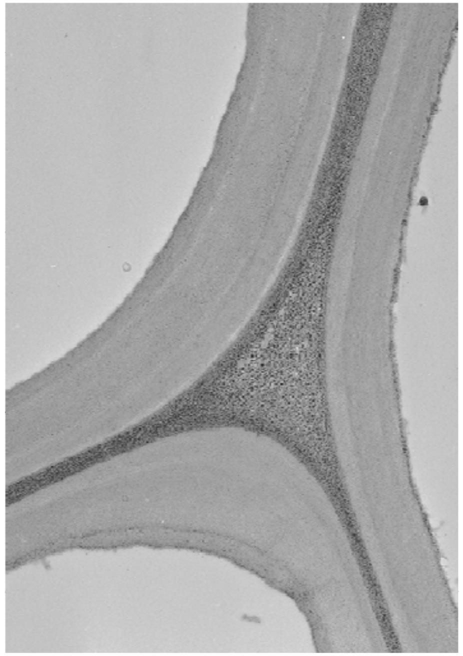
-Several scales of observation

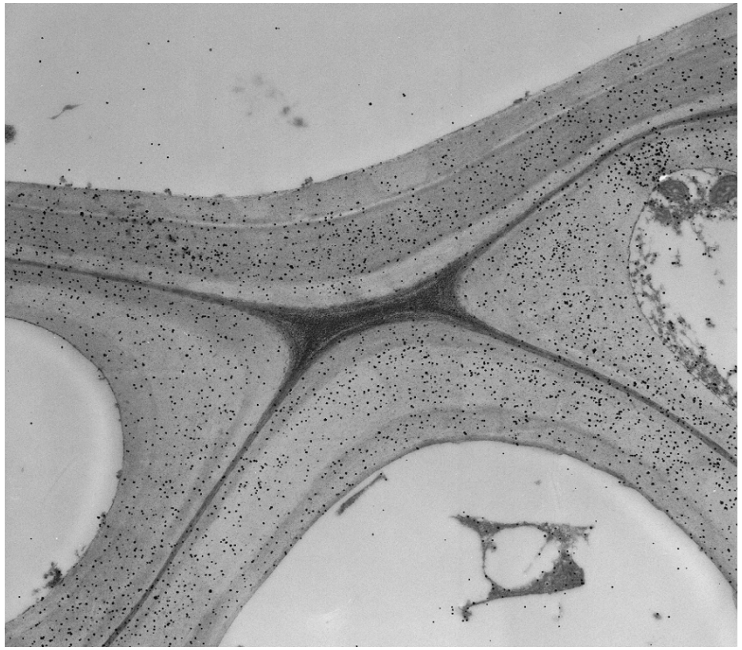
Principle of immunolabelling on ultrathin sections (for TEM)

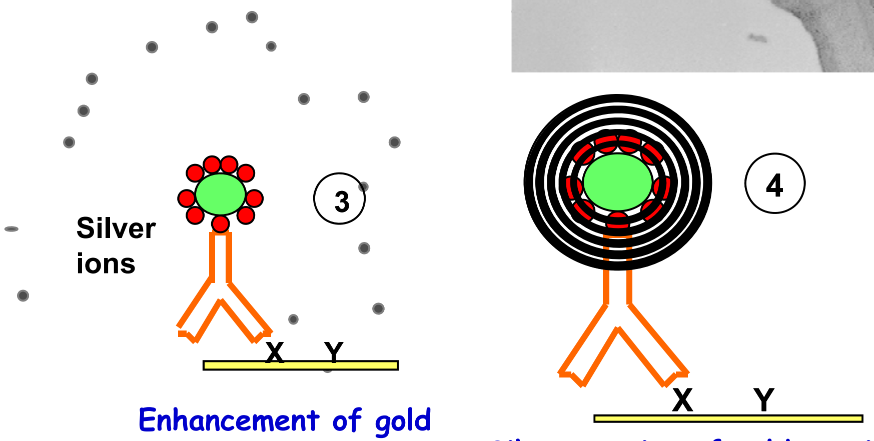


Antiserum containing IgGs









Enhancement of gold particles with silver ions

Silver coating of gold particles

The method is also available for light and other electron microscopies methods

In situ localization of matrix components

Xylans

Ruel et al.,2006, Maderas Cienc Technol
Billosta et al. 2006, Cellul Chem Technol
Joseleau and Ruel in New Knowledge in Wood Quality, K. Entwistle & J.C.F. Walker Eds., 2005,103-113

Kim et al.,Planta, 2010
Kim and Daniel, Planta, 2012
Koutaniemi et al., Planta, 2012

Interaction of xylans with Cellulose

In planta

Probes for Microscopy of Wood Cell Wall Hemicelluloses

Glyco-Hydrolases

endo-β(1,4)-xylanase	**Xylan**	Vian et al.	1983
endo-β(1,4)-mannanse	**Mannan**	Ruel & Joseleau	1984
β-D-galactosidase	**Xyloglucan**	Vian et al.	1991
α-D-glucosidase	**XG**	Ruel & Joseleau	1991
endo-β(1,4)-glucanase	**XG**	Vian et al.	1991
Cellobiohydrolase	**Cellulose**	Vuong et al.	1984

Antibodies against Hemicelluloses

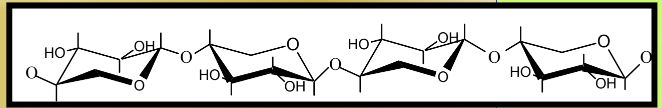
Anti-β-(1,4)-oligoXylose (P)	**β-Xylan**	Northcote et al.	1989
Anti-Xyloglucan (P)	**XG**	Moore et al.	1989
Anti-Xyloglucan (P)	**XG**	Joseleau et al.	1992
Anti-Xylan (P)	**GlucuronoXylan**	Awano et al.	1998
Anti-Glucomannan(P)	**GlucoMannan**	Maeda et al.	2000
BGM C6 (M)	**(1-4)-β-mannan**	Pettolino et al	2001
Anti- Xylan (P)	**Xylan-*l***	Ruel et al.	2004
Anti-Xylan (P)	**Xylan-*hs***	Ruel et al.	2004
CCRC-M1 (M)	**XG (Fuc*t*)**	Puhlmann et al.	1994
LM 10 (M)	**Xylan-*l***	McCartney et al.	2005
LM 11 (M)	**ArabinoXylan**	McCartney et al.	2005
LM 21, LM 22 (M)	**glucomannan**	Marcus et al.	2010
collection of several new Mabs	**glycan epitopes**	**Pattathil et al.**	2010
Substituent Specific glucuronoxylans		Koutaniemi at al.	2012

Carbohydrate-Binding Modules

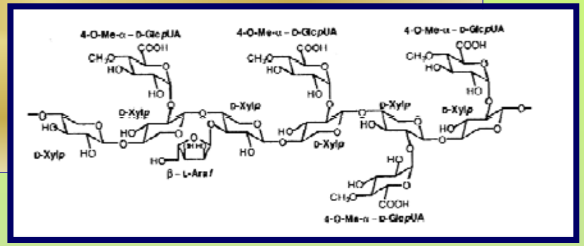
Xylan CBM (35 CBMs)	**Various Xylans**	McCartney et al.	2006

Two types of Xylans:

- Low substitution pattern



- High substitution pattern



Preparation of **two antibodies against:**

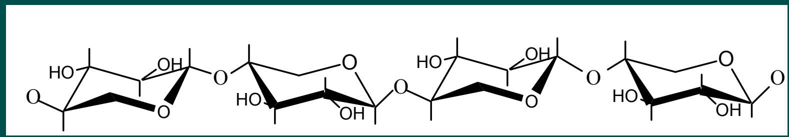
Unsubstituted Xylans → **Anti-X *l***

Highly substituted Xylans → **Anti-X *hs***

TEM

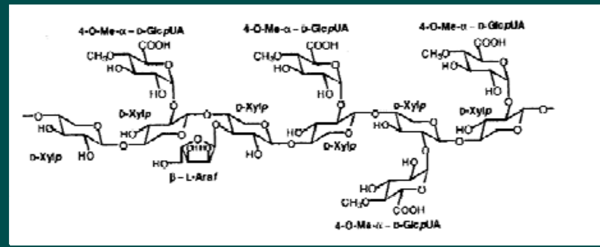
Distribution of Xylans in Poplar Wood

linear xylan

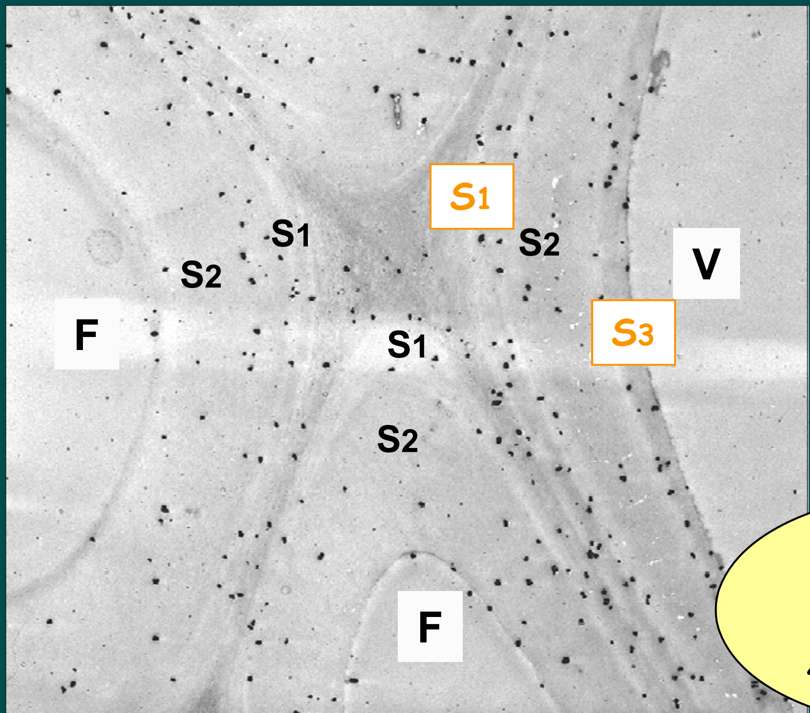


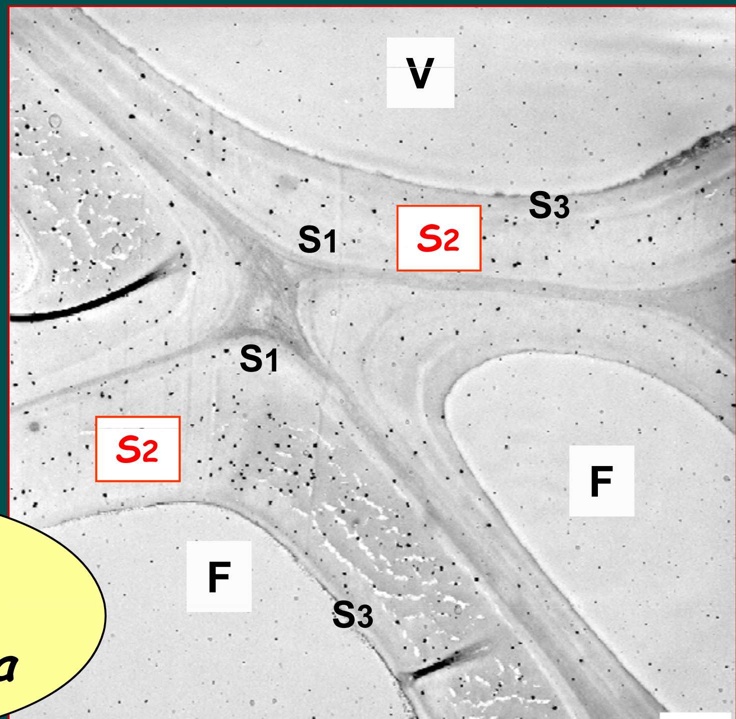
Anti-X *l*

highly substituted



Anti-X *hs*



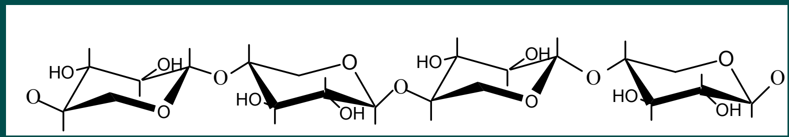


Populus
Tobacco
A. thaliana

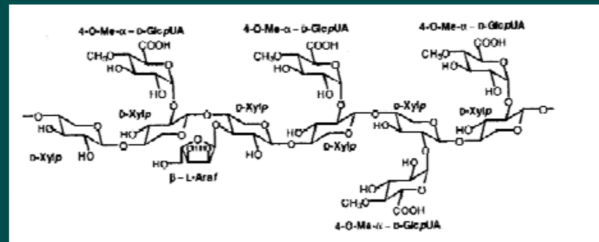
TEM

Distribution of Xylans in Poplar Wood

linear xylan



highly substituted



⇨Linear and substituted Xylans have a specific topochemical distribution at the nanoscale

⇨ Distribution of linear and substituted xylans is spatio-temporally controlled

⇨This distribution is the same in cell walls having the same mechanical function

Glucomannans

in **Conifer** tracheids

- Mishima et al., 1998; Hannuksela et al., 2002
- Maeda et al., *Protoplasma,* 2000
- Hosoo et al., *Planta,* 2002
- Awano et al., *Protoplasma*, 2002
- Altaner et al., *BioResources*, 2007
- Prislan et al., *Holzforschung*, 2009
- Stevanic and Salmen, *Holzforschung*, 2009
- Salmen and Burgert, *Holzforschung*, 2009
- Kim et al., *Planta*, 2010, 2011
- Kaneda et al., *JIPB*, 2010
- Kim and Daniel, *Planta*, 2012
- Donaldson and Knox, *Plant Physiol*., 2012

☛ **localized to the secondary wall of tracheids with increased deposition at the S1/S2 boundary**

☛ **localized in close association with cellulose**

☛ **deposited prior to lignification**

Glucomannans have in Softwood a role similar to that of Xylans in Hardwood