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PhD student
under the direction of Fabio Gennaretti

Dendroecology course

Quantitative Wood Anatomy

UQAT

GREMA

Chaire Dendro-eco

Wood Anatomy Basics

Wood = 3-dimensional tissue

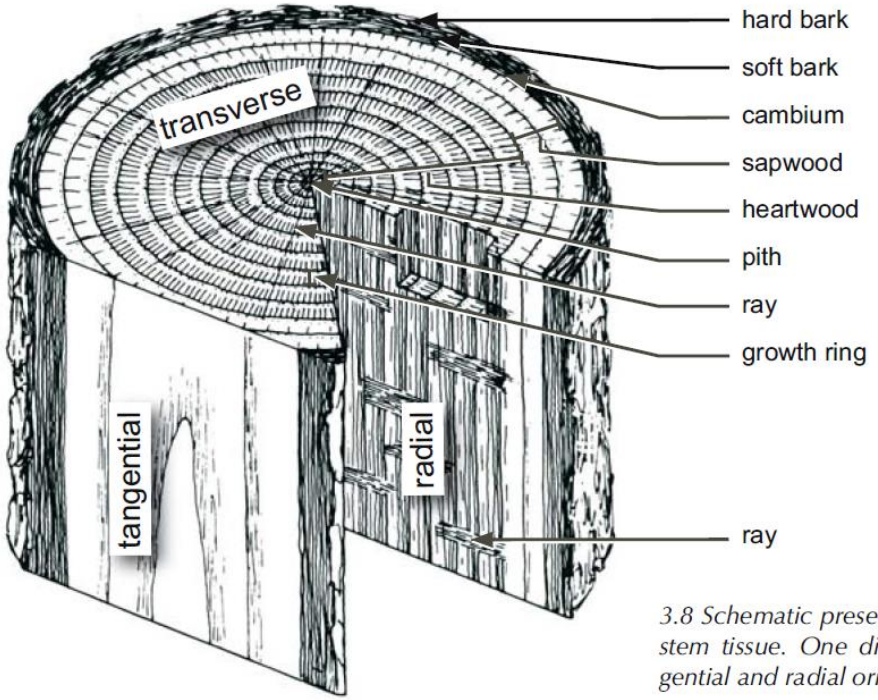
Wood is :

→ Heterogeneous,

→ Hygroscopic (attract & hold water molecules),

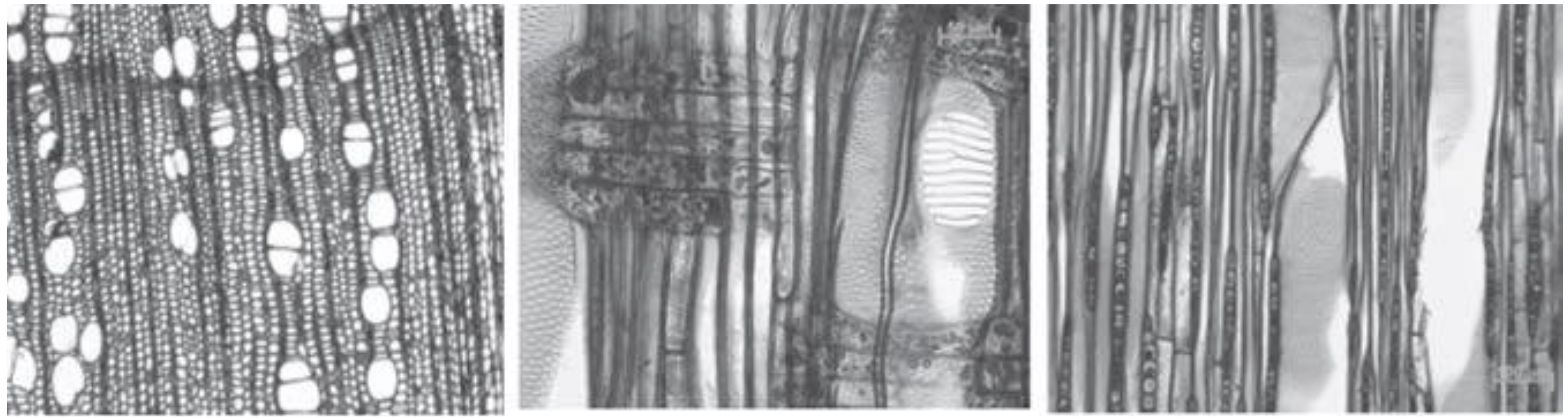
→ Cellular (cells are the basic structural and functional units),

→ Anisotropic (or orthotropic, non-uniform in different directions)

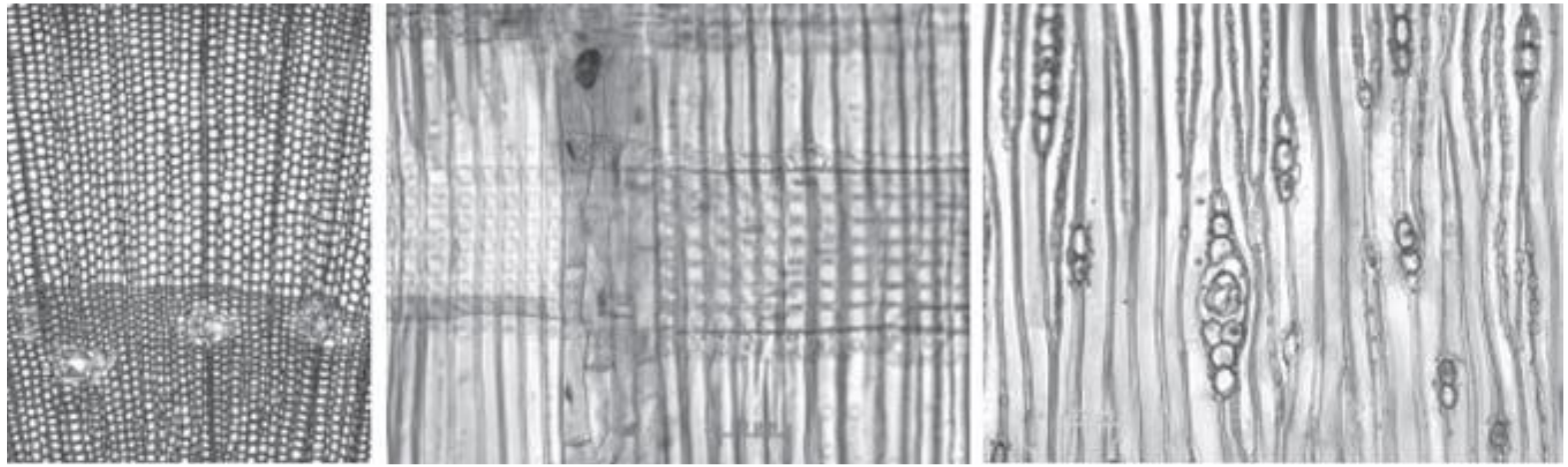


3.8 Schematic presentation of the principal orientation of stem tissue. One distinguishes between transverse, tangential and radial orientation (Schweingruber 1978).

Angiosperms : *Alnus nepalensis*



Conifers : *Pinus sylvestris*

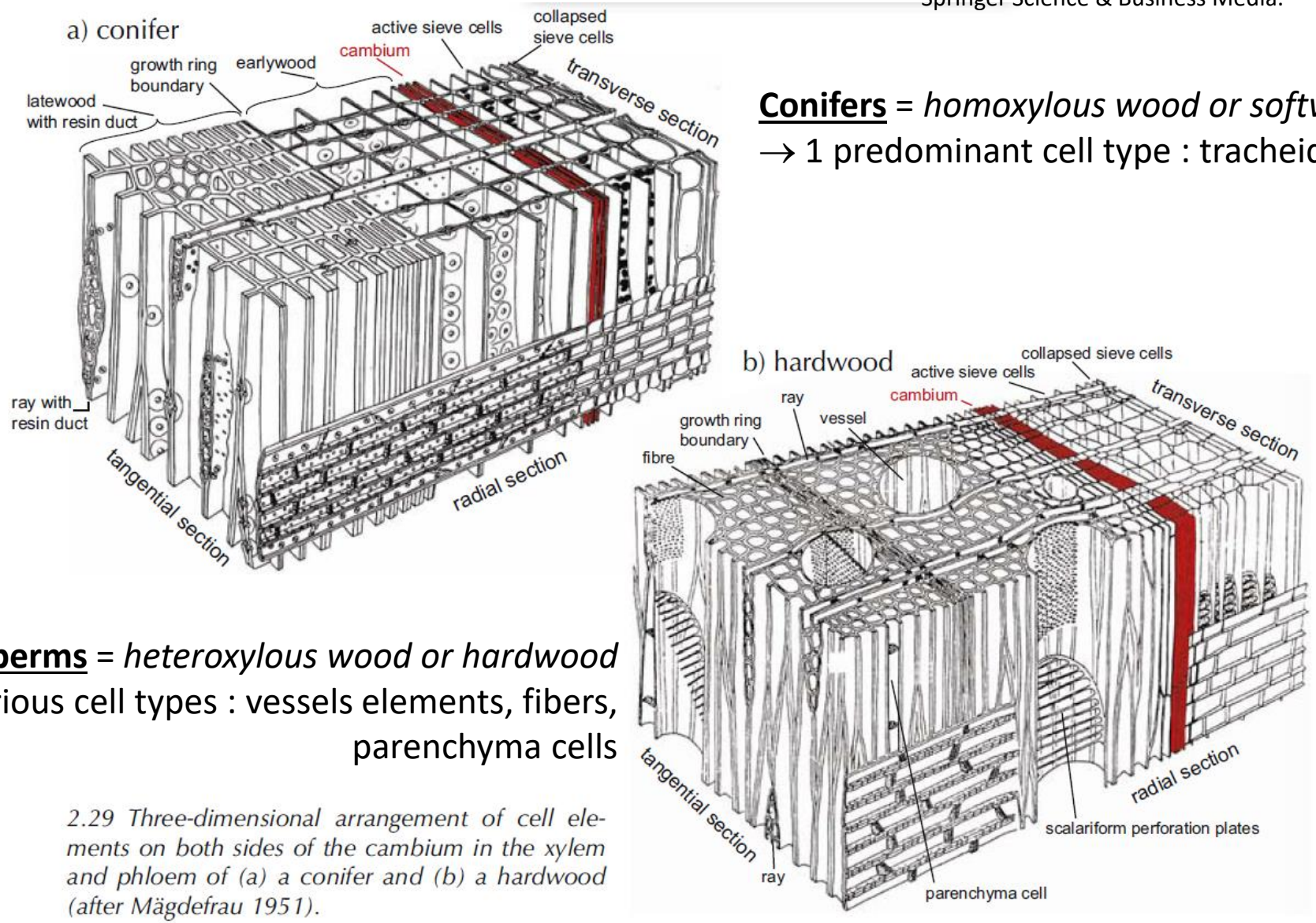


From Cutler, D. F., Botha, T., & Stevenson, D. W. (2008). Plant anatomy. *An applied approach. Malden, MA: Blackwell Publishing*.

Wood Anatomy Basics

Angiosperms vs. Conifers

Schweingruber, F. H., Börner, A., & Schulze, E. D. (2007). *Atlas of woody plant stems: evolution, structure, and environmental modifications*. Springer Science & Business Media.



Conifers = *homoxylous wood or softwood*

→ 1 predominant cell type : tracheids (~90%)

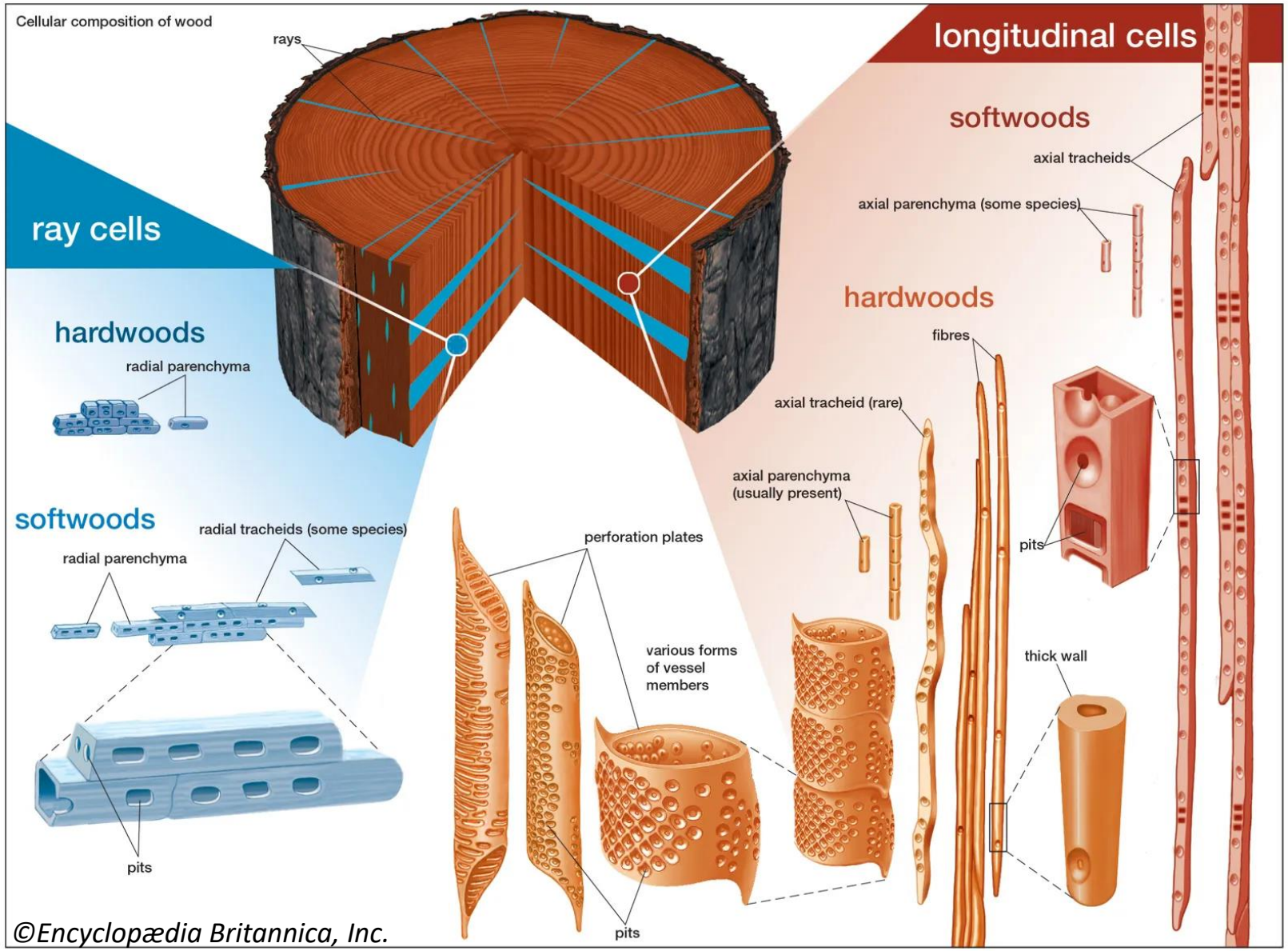
Angiosperms = *heteroxylous wood or hardwood*

→ various cell types : vessels elements, fibers, parenchyma cells

2.29 Three-dimensional arrangement of cell elements on both sides of the cambium in the xylem and phloem of (a) a conifer and (b) a hardwood (after Mägdefrau 1951).

Wood Anatomy Basics

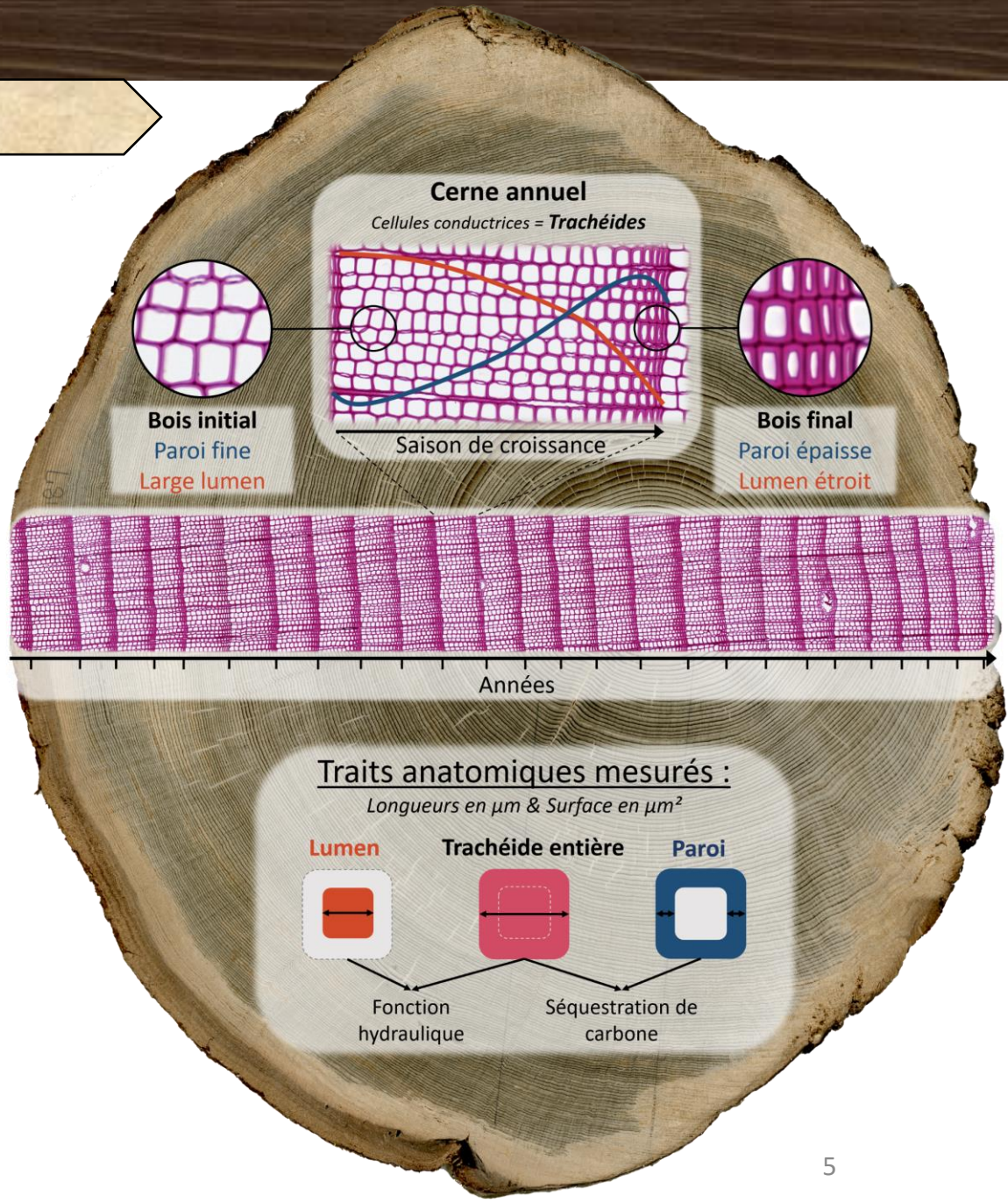
Wood cells



Quantitative Wood Anatomy

Anatomical traits

- QWA = “numeric analysis of xylem (or wood) anatomical traits (WAT) of plants and their relationship to plant functioning, growth, environment, wood quality and species identification” (Von Arx, 2021)
- **Fine temporal resolution :** Inter-annual to subseasonal
- Large number of measurable traits
- Each trait is specifically influenced by the environment
- Direct **Structure-Function** relationships

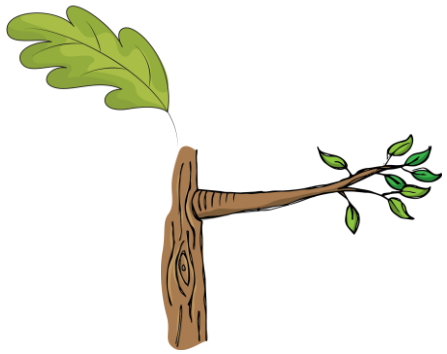


Quantitative Wood Anatomy

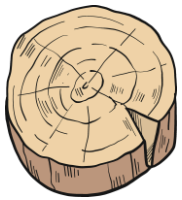
Anatomical traits

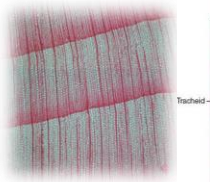
- All **measurements** of :
  - Dimensions (diameter, area…)
  - Quantities (counts, abundance…)
  - Proportions (tissue %…)
  - Absence/presence of a structure
  - Positions & spatial arrangement

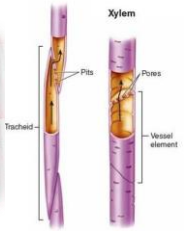
- Xylem is present in :
  - Roots
  - Stems
  - Branches
  - Leaf veins (primary xylem)

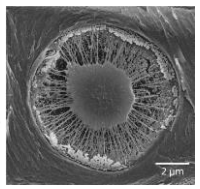


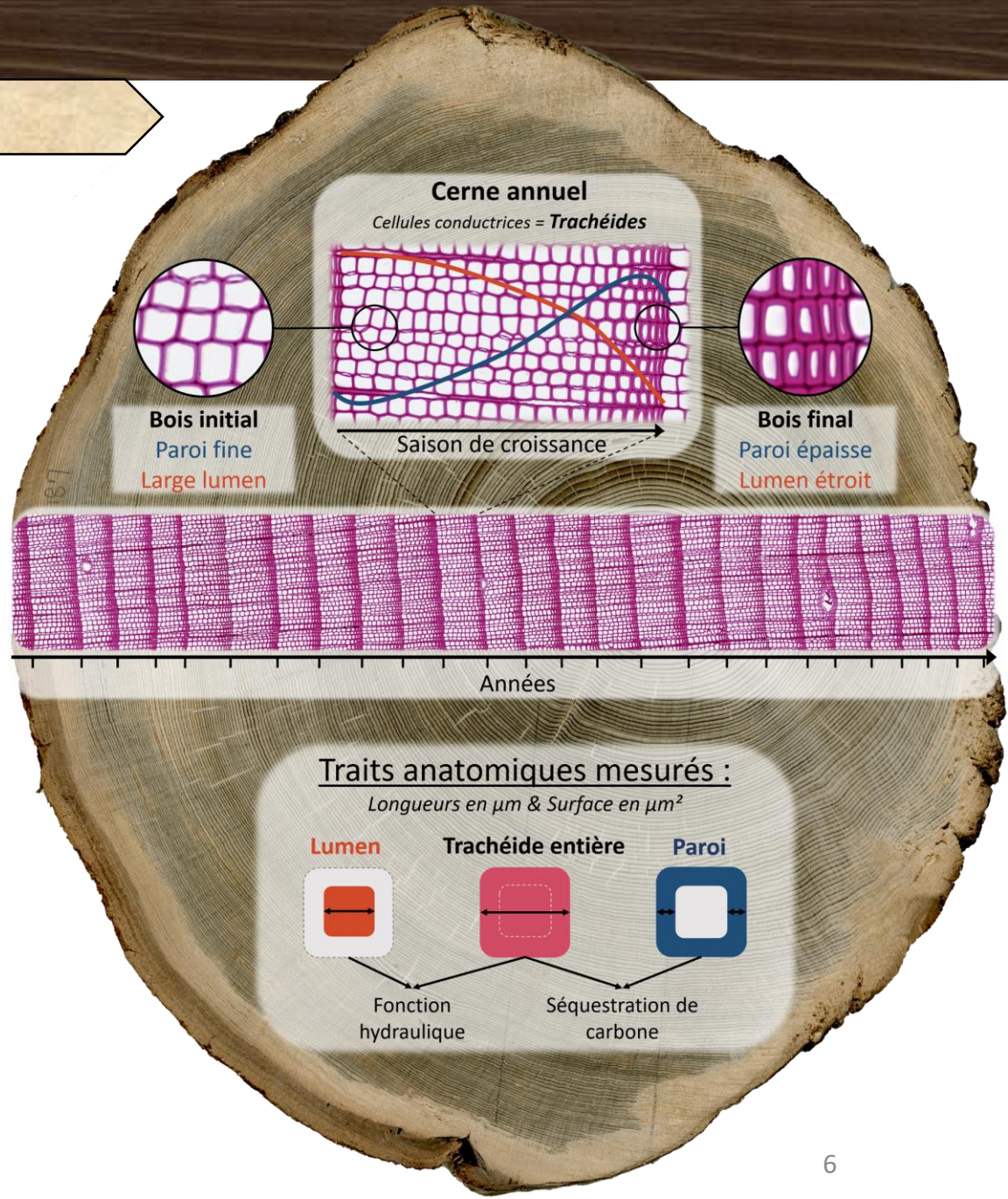
- At the **scale** of :
  - Organs & tissues
  - Growth rings
  - Constitutive cells
  - Sub-cellular structures (ex : pits)









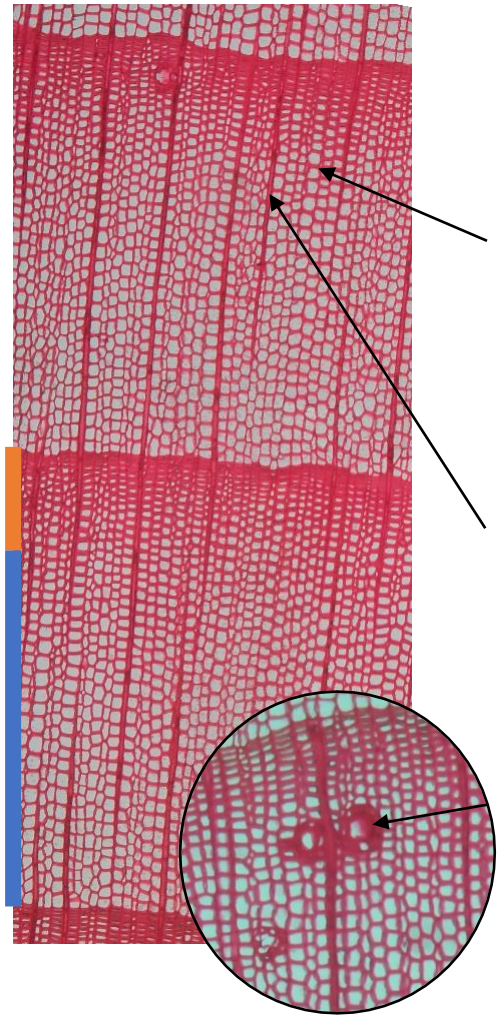


Quantitative Wood Anatomy

Anatomical traits

Examples of measured wood anatomical traits in a transverse section

Picea mariana



Conifers

Tracheids
Lumen diameter
Cell Wall Thickness

Rays
Width
Density

Resin ducts
Number
Position

Latewood
Earlywood

Angiosperms

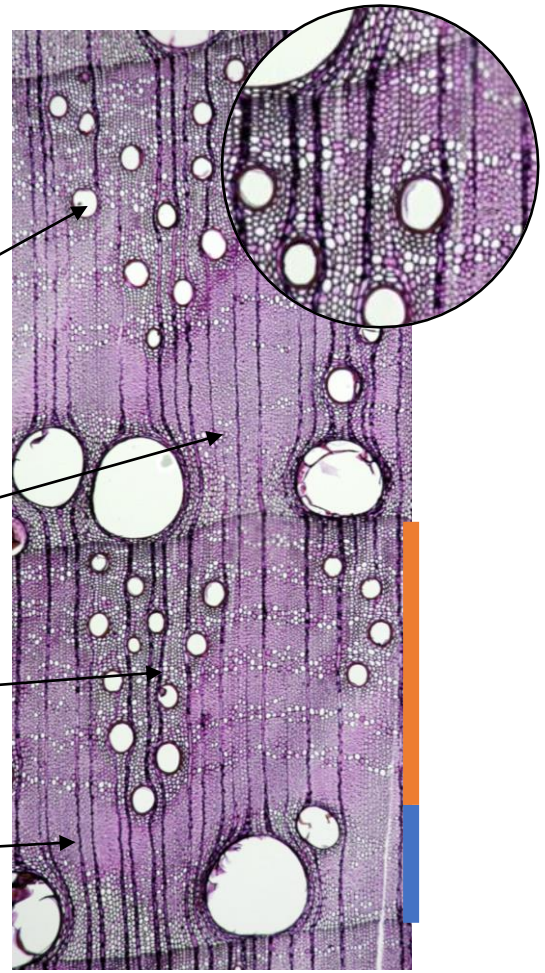
Vessels
Size
Roundness
Grouping

Fibers
Cell Wall Thickness
Tissue Proportion

Axial parenchyma
Tissue proportion

Rays
Width
Density

Quercus acutissima

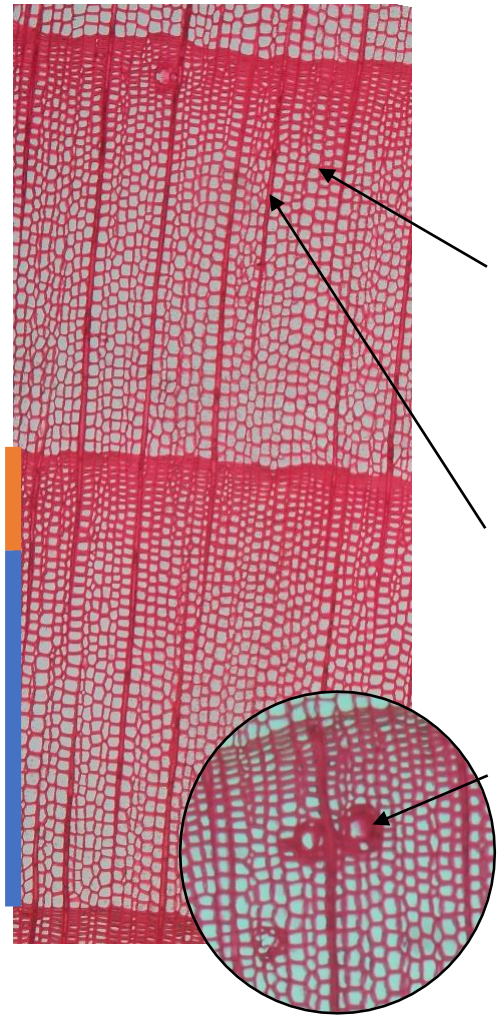


Quantitative Wood Anatomy

Structure/Function relationships

Wood anatomical traits as indicators of functional properties

Picea mariana



Conifers

Tracheids
Water transport
Mechanical support
Carbon sequestration

Rays
Storage
Radial transport

Resin ducts
Defense

Latewood
Earlywood

Angiosperms

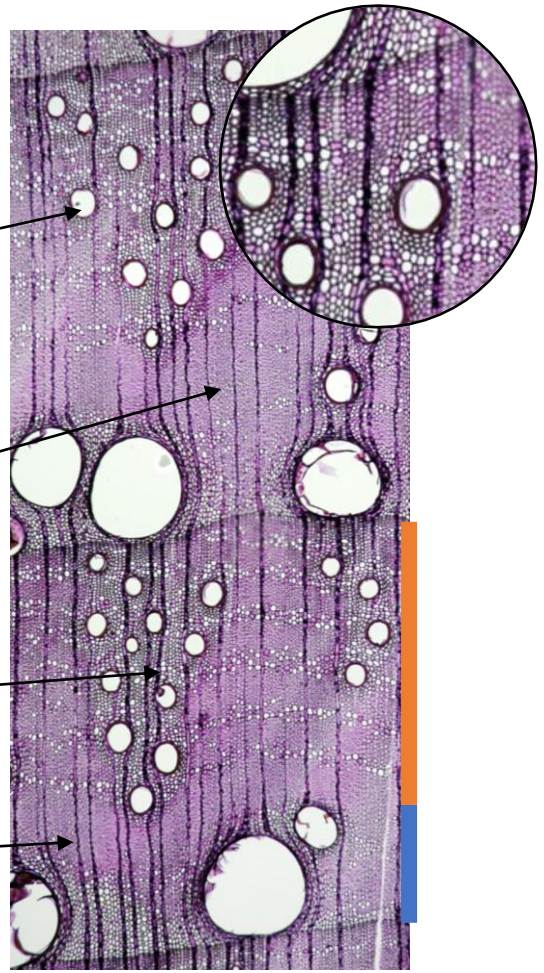
Vessels
Water transport

Fibers
Mechanical support
Carbon sequestration

Axial parenchyma
Storage

Rays
Storage
Radial transport

Quercus acutissima



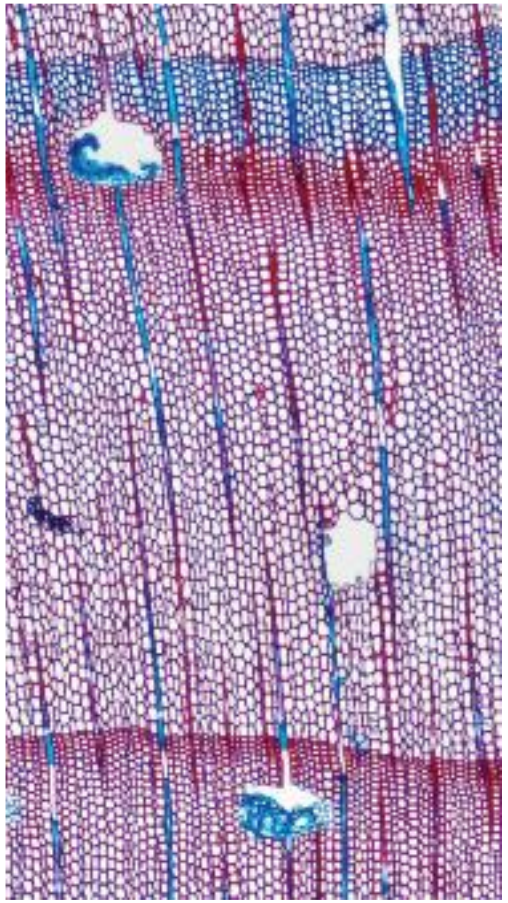
©Insidewood Images

Quantitative Wood Anatomy

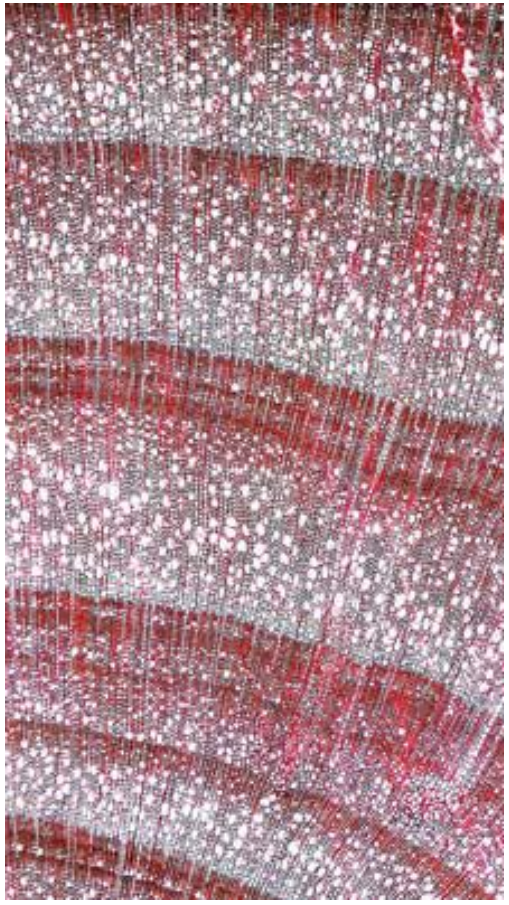
Discrete Wood Anatomical Features

Crivellaro et al. (2018), Schweingruber (2008), Montwé et al. (2018), Stoffel et al. (2014)

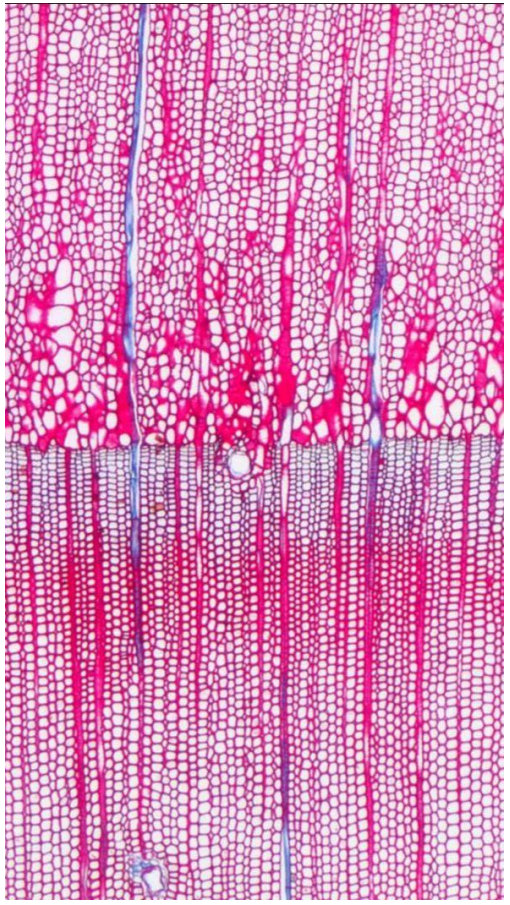
Examples of discrete wood anatomical features as reactions to environmental stresses



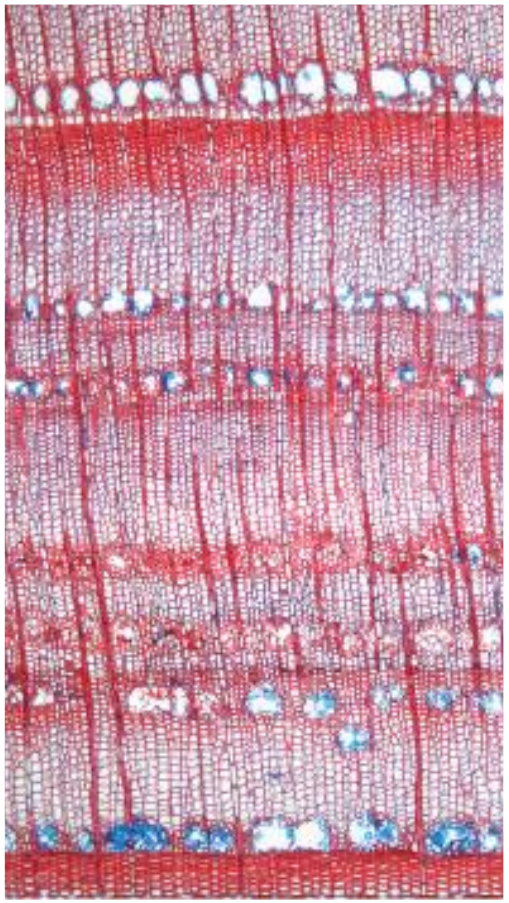
Blue rings & Light rings



Intra-annual density fluctuations



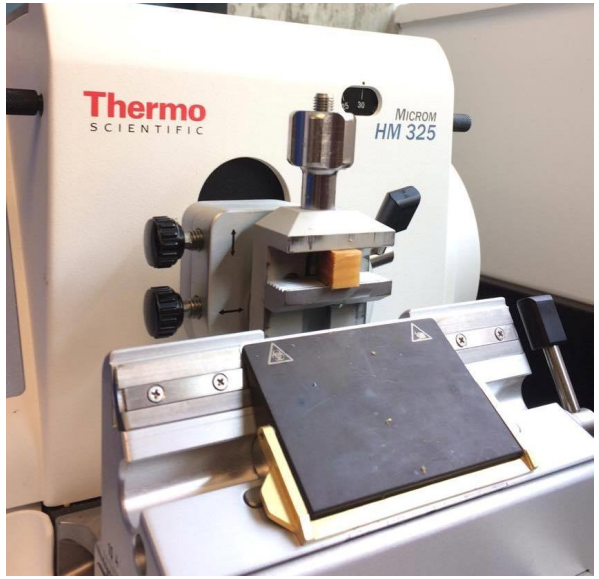
Frost rings



Rows of traumatic resin ducts

Quantitative Wood Anatomy

Lab methods



Step 1 : Cutting with a Microtome → thin sections of 6 to 20 µm

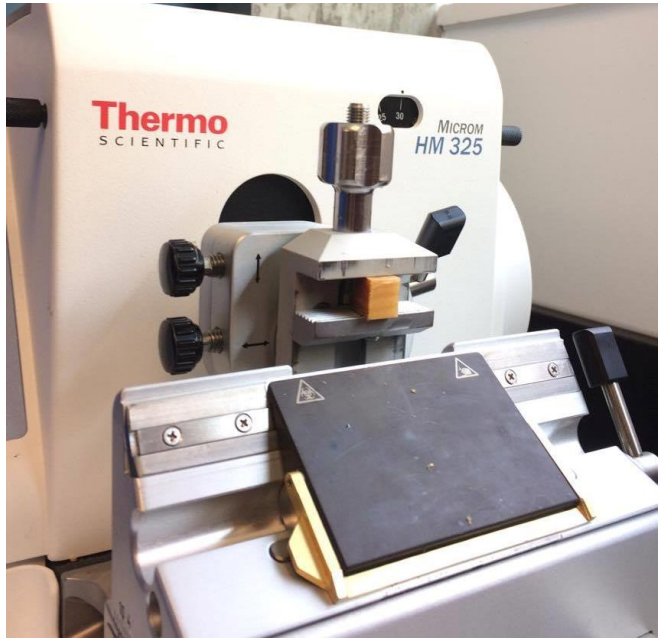
Step 2 : → Dehydration with increasing concentrations of alcohol,
→ staining to increase contrast,
→ mounting of permanent slides

Step 3 : Image Numerization
→ Slide scanner
→ Camera with microscope + image stitching

Get the best image quality possible !

Quantitative Wood Anatomy

Lab methods



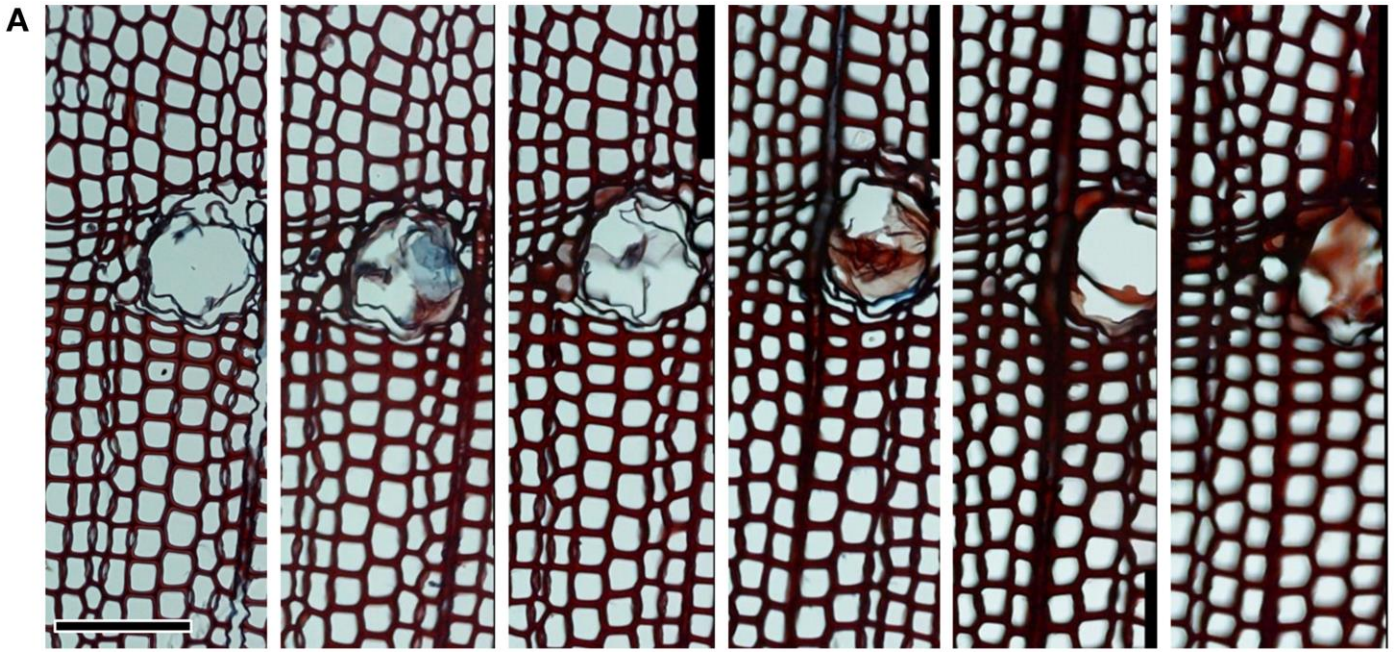
Step 1 : Cutting with a Microtome

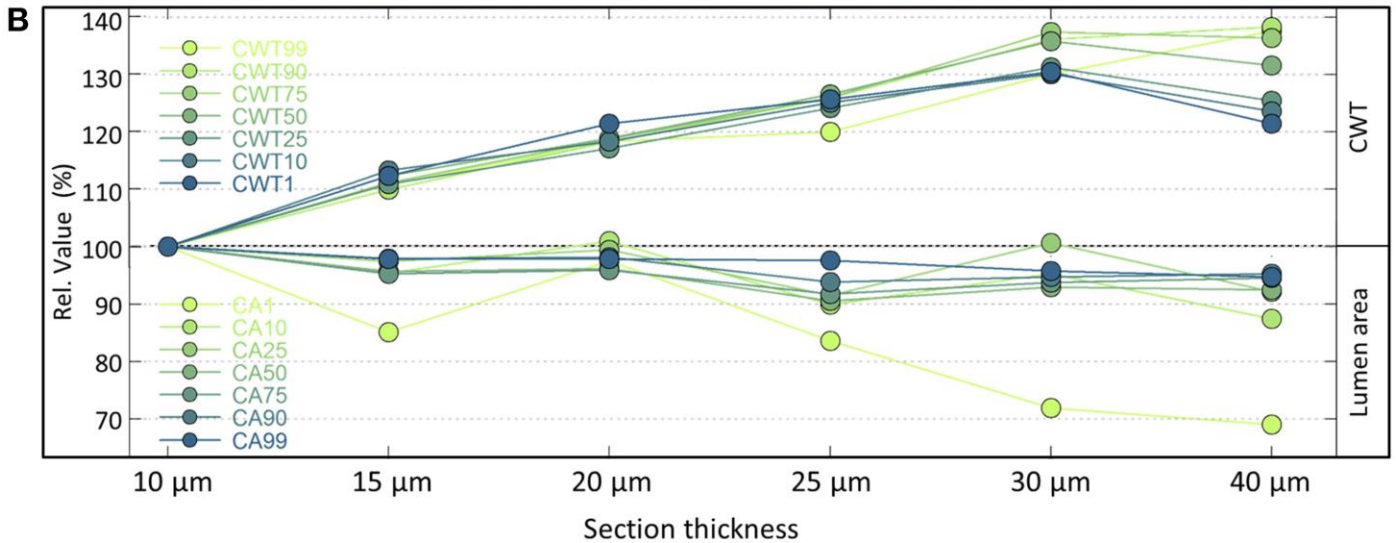
Sources of bias :

→ Section thickness

→ Sample orientation

→ Blade quality



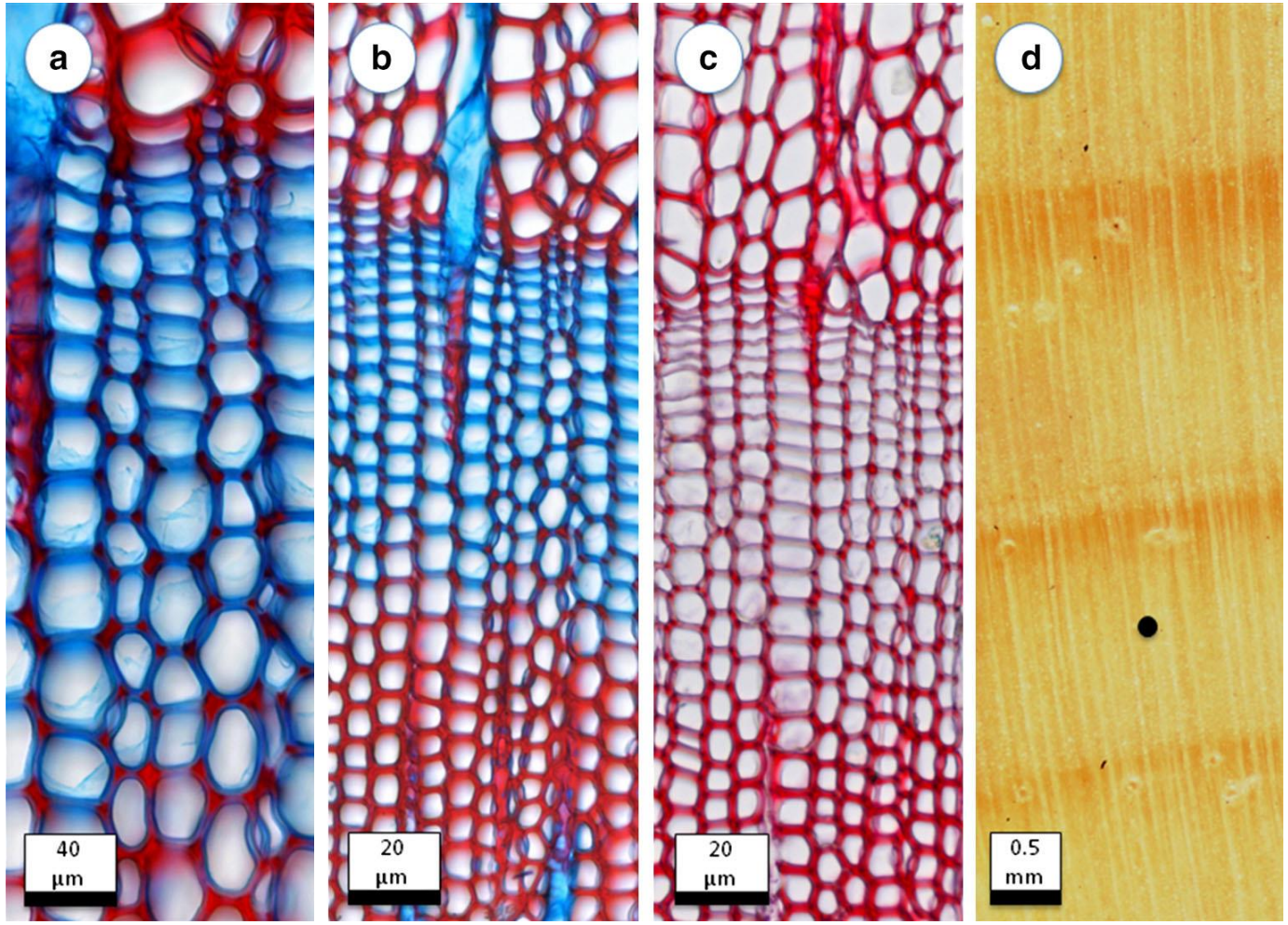


Von Arx, 2016

Quantitative Wood Anatomy

Lab methods

Staining



Piermattei, 2015

The staining technique depends on what we want to see

Ex : Blue rings in conifers

a – b) Double staining with **Safranin + Astrablue**
→ non-lignified cells are stained in blue

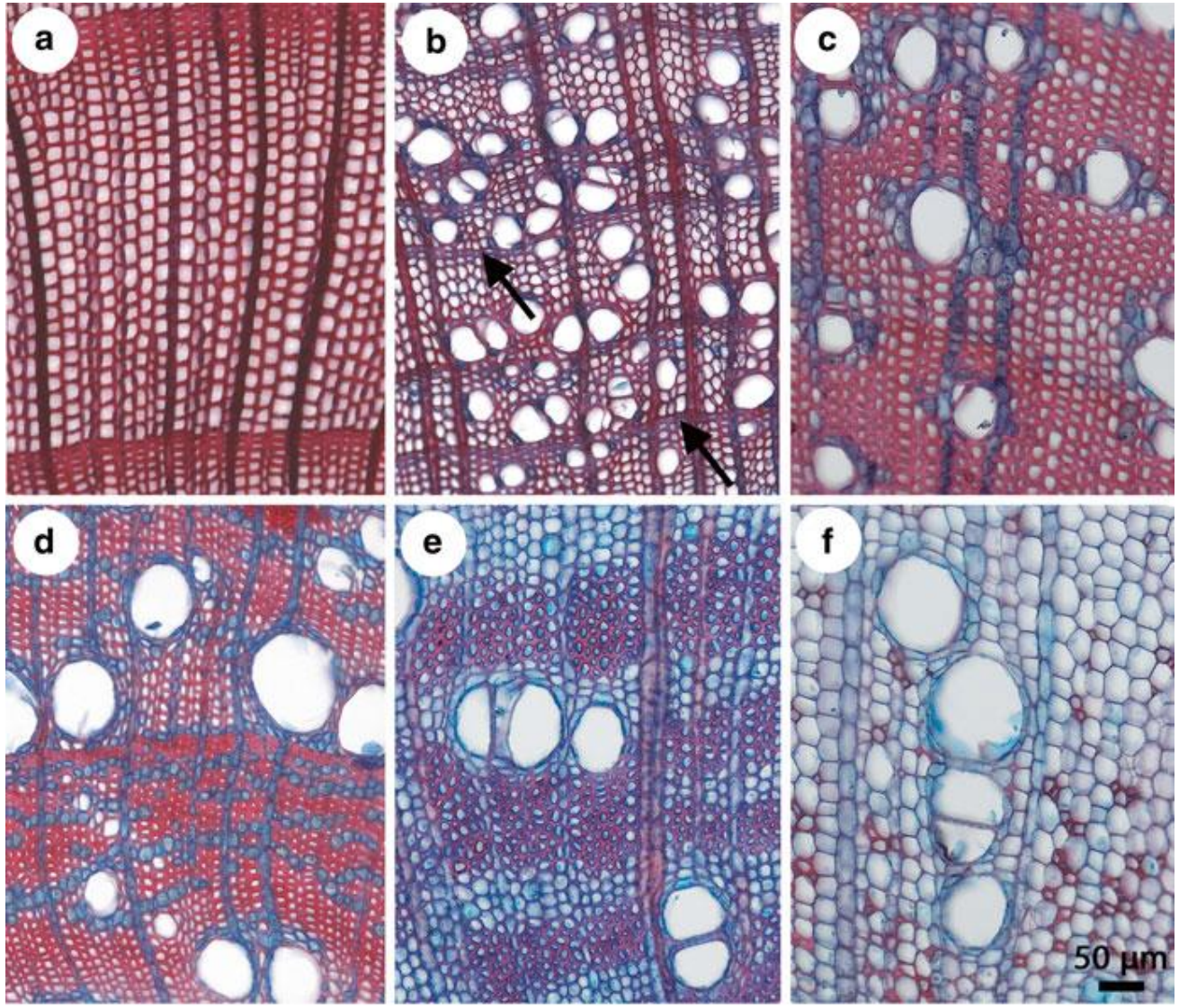
c) Simple Safranin staining

Quantitative Wood Anatomy

Lab methods

Plavcová, L., Jansen, S. (2015). The Role of Xylem Parenchyma in the Storage and Utilization of Nonstructural Carbohydrates

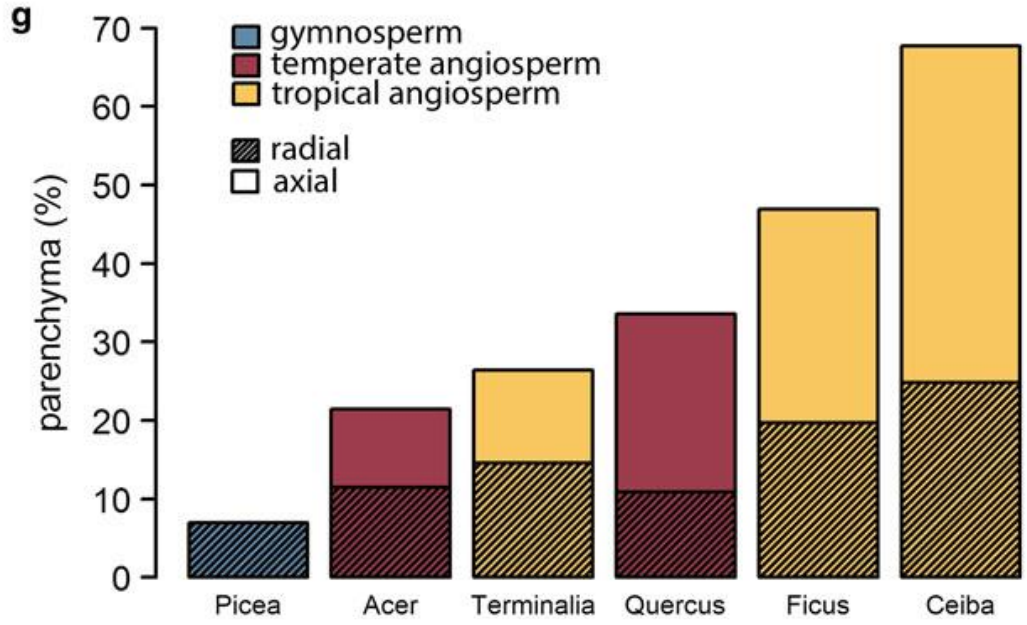
Staining



The staining technique depends on what we want to see

Ex : Parenchyma

a – f) Double staining with **Safranin + Alcian blue**
→ Protoplasts of parenchyma cells are blue



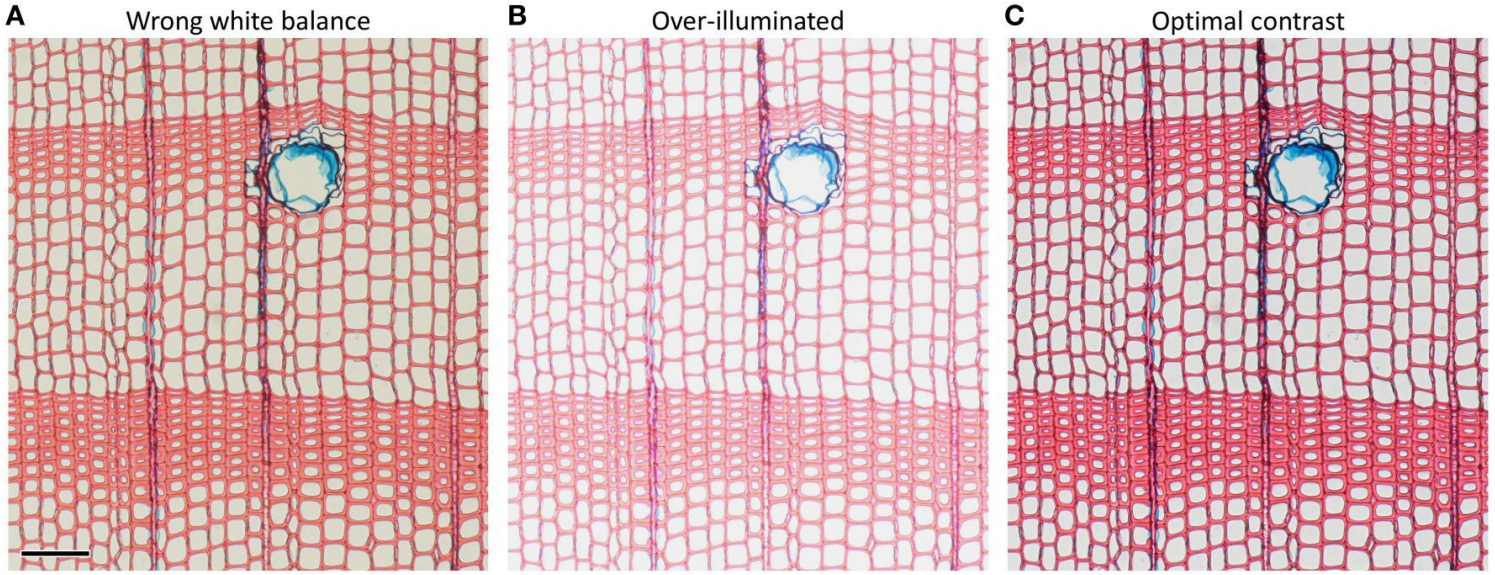
Quantitative Wood Anatomy

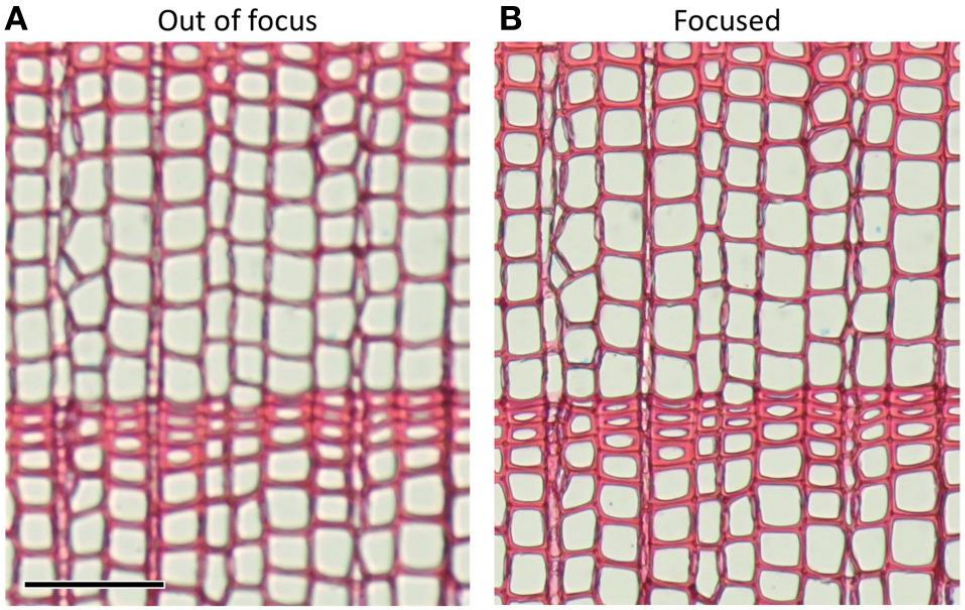
Lab methods

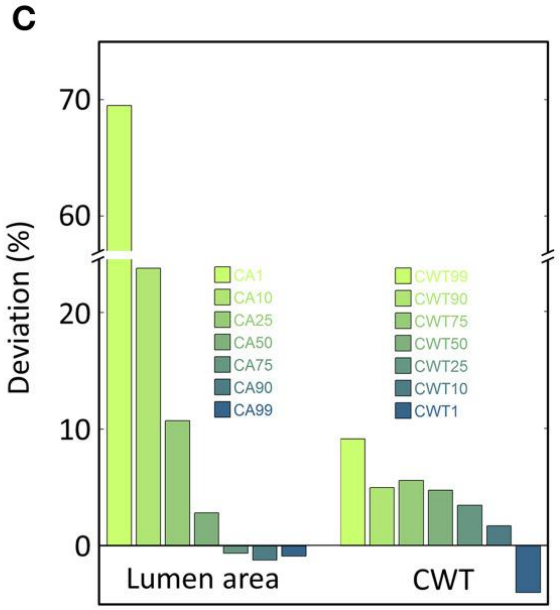
Step 3 : Image Numerization

→ 10x objective are usually sufficient
Giving a resolution of 1.7–2.5 pixels/µm

→ 4x objective can be used for angiosperm vessels







Von Arx, 2016

Quantitative Wood Anatomy

Image Analysis Software & Tools

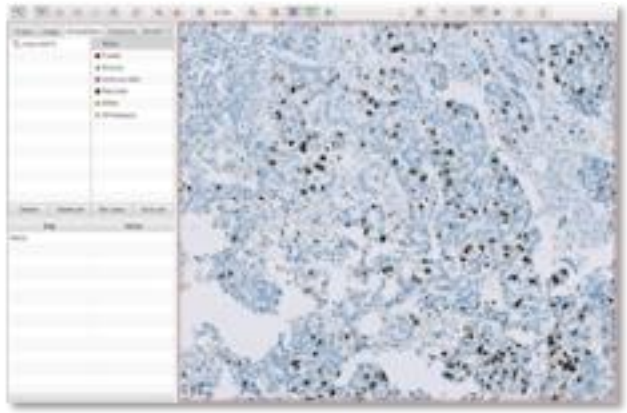
Step 4 : Image Analysis

Specialized Wood Anatomy Softwares	Image Analysis with Graphical User Interface	Programming Language Libraries
WinCELL™ Wood Cell Anatomy	QuPath Quantitative Pathology & Bioimage Analysis	NumPy
ROXAS	CellProfiler™ cell image analysis software	SciPy
	Fiji/ImageJ	OpenCV
		scikit-image image processing in python

Quantitative Wood Anatomy

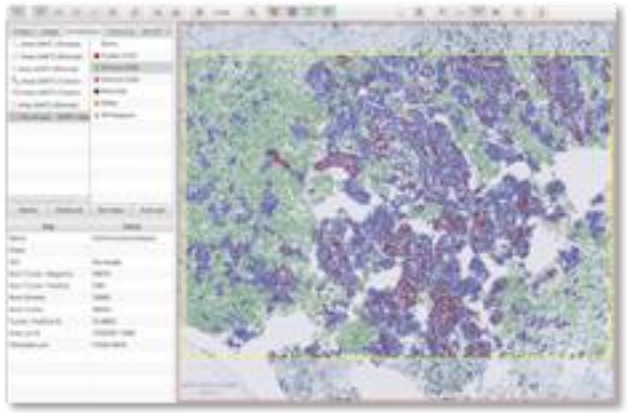
Image Analysis Basics

Step 4 : Image Analysis



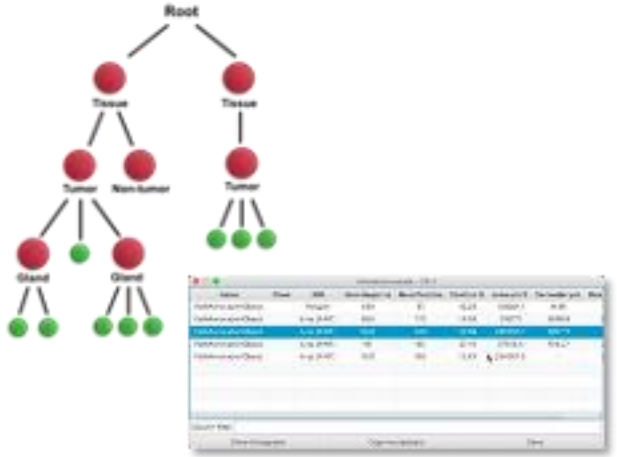
Start with pixels

Billions of numbers



Identify objects

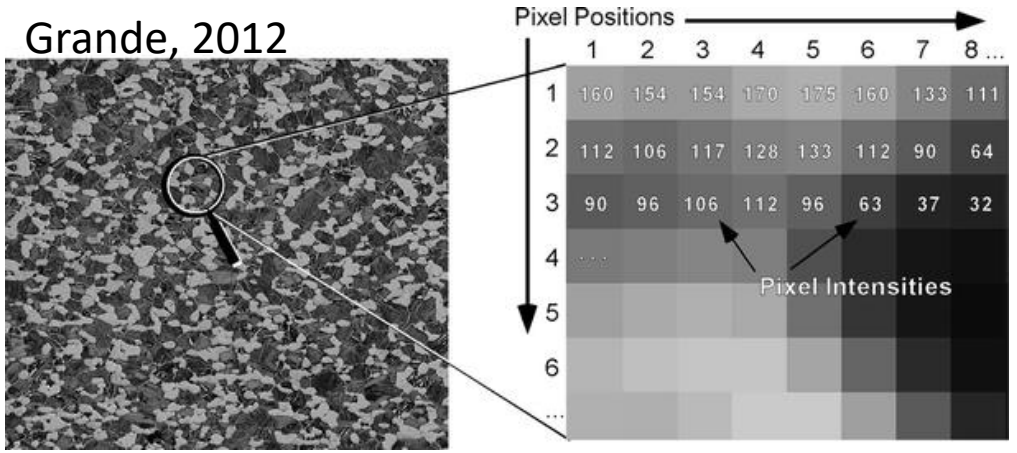
Cells, structures, regions…

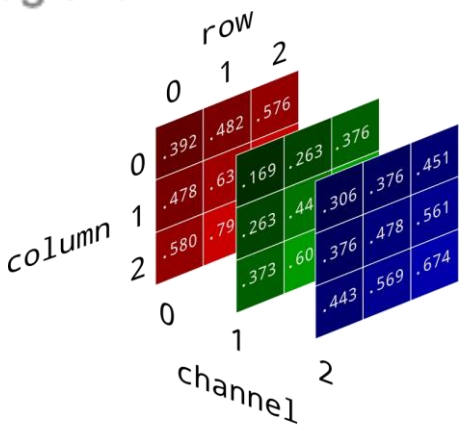


Query the objects

Size, shape, number, intensity, location, classification…

Grande, 2012





Images are 2- to multi-dimensional arrays (or matrices) of pixels associated with values

Quantitative Wood Anatomy

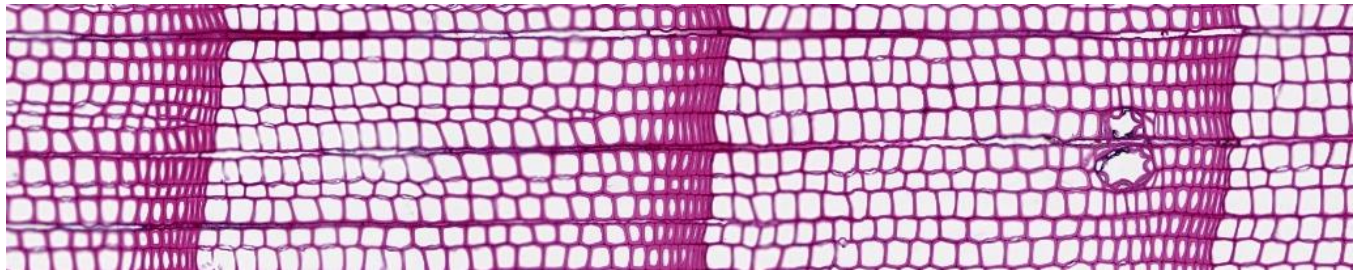
Image Analysis Workflow

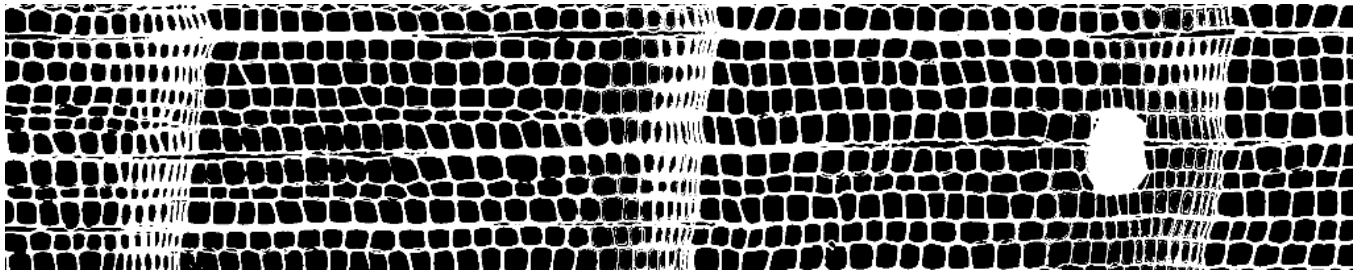
Thresholding
Binarization

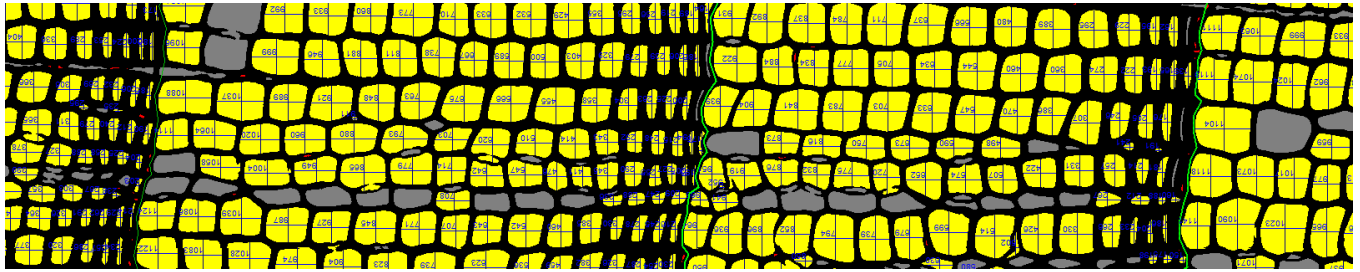
Segmentation/Labelling
Object detection

Object measurements

Numerical data:
Shape, Size, Location informations for each cell







Cell#	Group#	Type	LumenArea	LumenLength	LumenWidth	HorizontalPosition	VerticalPosition	LeftWallLength
1	0	C	25,4495	6,6051	4,4034	5834,7349	1803,0675	0,5504
2	0	C	17,8752	3,3026	6,0547	5833,1818	1811,8468	0,5504
3	0	C	47,8692	3,3026	14,8615	5834,3859	1826,6544	1,6513
4	0	C	6,9683	1,6513	4,9538	5833,5201	1785,561	0,8256
5	0	C	5,7564	1,6513	3,853	5833,4823	1793,7833	0,8256

Quantitative Wood Anatomy

Image Analysis Basics

Guidelines for Best Practices in Image Processing

→ When it's possible : **avoid image edition**

Treating Images as Data: Digital scientific images should be treated as data

Filters Degrade Data: Use of software filters to improve image quality is usually not recommended for biological images.

Comparing Images: Digital images that will be compared to one another should be acquired under identical conditions.

Saving the Original: Manipulations of digital images should always be done on a copy of the raw image data. The original must be retained.

Cloning Degrades Data: Cloning objects into an image or from other parts of the image is very questionable.

Manipulating the Entire Image: Manipulations that are specific to one area of an image and are not performed on other areas are questionable.

Making Simple Adjustments: Simple adjustments to the entire image are usually acceptable.

Making Intensity Measurements: Intensity measurements of digital images should be performed on raw data and the data should be calibrated to a known standard.

Issues With Magnification: Magnification and resolution issues are important.

Cropping is usually OK: Cropping an image is usually acceptable.

Lossy Compression Degrades Data: Avoid the use of lossy compression.

Issues With Pixels: Be careful when changing the size (in pixels) of a digital image.

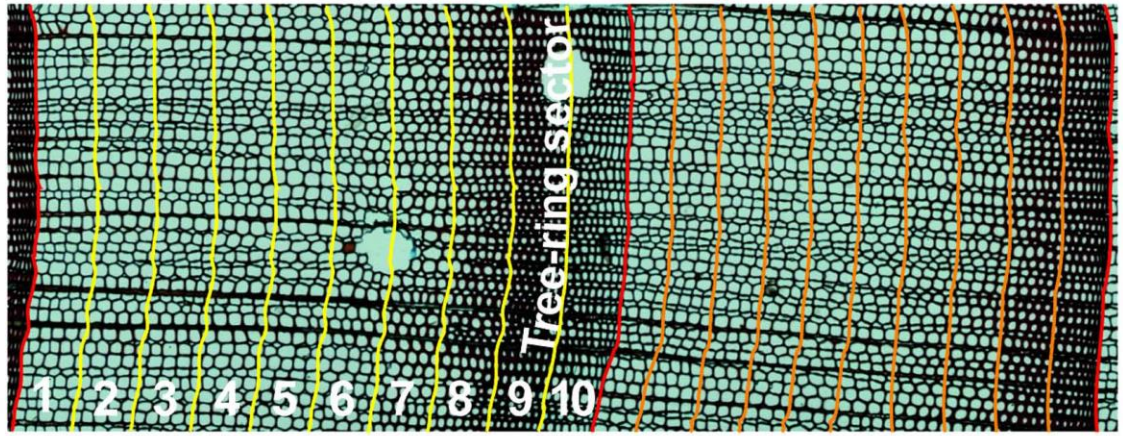
https://ori.hhs.gov/education/products/RIandImages/default.html

Quantitative Wood Anatomy

Data analysis methods

Tree-ring sectorization & Tracheidograms

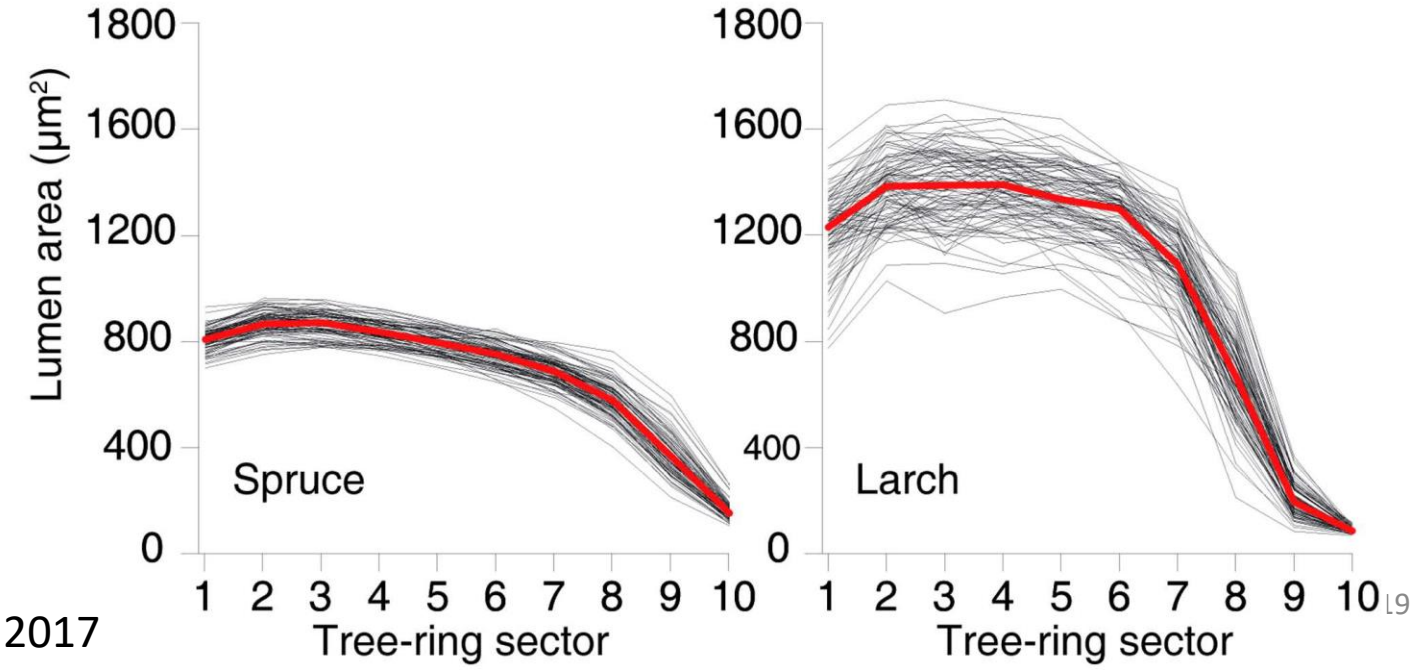
<u>How to get tree-ring anatomical structure at the intra-annual scale ?</u>



Method 1 :

Ring divided in a fixed number of sectors of equal width

→ Sectors of different rings have different widths



Carrer, 2017

Quantitative Wood Anatomy

Data analysis methods

Tree-ring sectorization & Tracheidograms

<u>How to get tree-ring anatomical structure at the intra-annual scale ?</u>

Method 2 :

Rings are divided in sectors of equal width

→ Differents rings can have a different number of sectors

Bjorklund, 2020

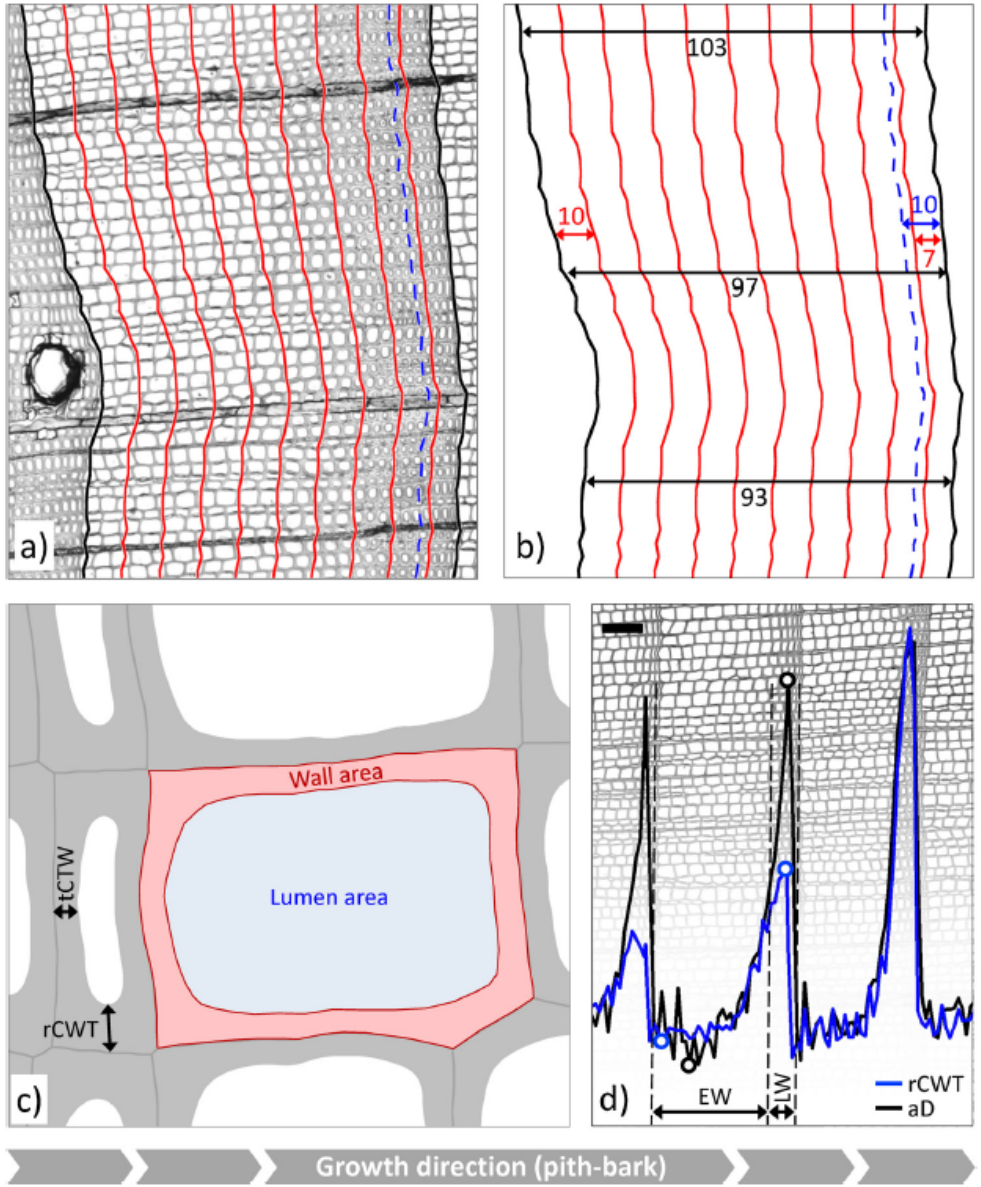


Fig. 1. Explanatory figure of measured parameters and measurement technicalities. a) and b) Visualization of the algorithm used to create intra-annual profiles of anatomical parameters based on cell position in the ring. Cells are here assigned to on average 10 unit wide bands (in the analysis we use 10 µm bands) (red lines) parallel to the ring borders (black lines) based on their center coordinates. Anatomical parameters are then calculated as the 75th percentile in each band. Bands are not allowed to cross ring borders and thus never include cells from two adjacent rings. To avoid a truncated narrower last band towards the ring borders (7 units in the b) example), the terminal band (blue dashed line) is defined as the 10 units adjacent to the terminal ring border. This means that cells in the overlapping part of the last and second-last band are included in both bands. c) Illustration of the basic tracheid dimensions used in this study: rCWT – radial cell wall thickness (i.e., the walls running in radial direction in a cross-sectional view), tCWT – tangential cell wall thickness (walls running in tangential direction in a cross-sectional view), lumen area and wall area. Anatomical density (aD) of each cell is defined as the ratio of wall area to overall cell area (sum of wall and lumen area). d) Exemplary 10-µm-resolution intra-annual profiles for rCWT (blue curve) and aD (black curve). Maximum and minimum values for each parameter (circles) are extracted for each ring, whereas means for earlywood (EW) and latewood (LW) are obtained by averaging the values of the bands assigned to EW and LW, respectively. Scale bar in (d): 100 µm (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Quantitative Wood Anatomy

Data analysis methods

Tree-ring sectorization & Tracheidograms

How to get tree-ring anatomical structure at the intra-annual scale ?

Method 3 :

Vaganov standardization :

→ All radial files are standardized to a fixed number of tracheids

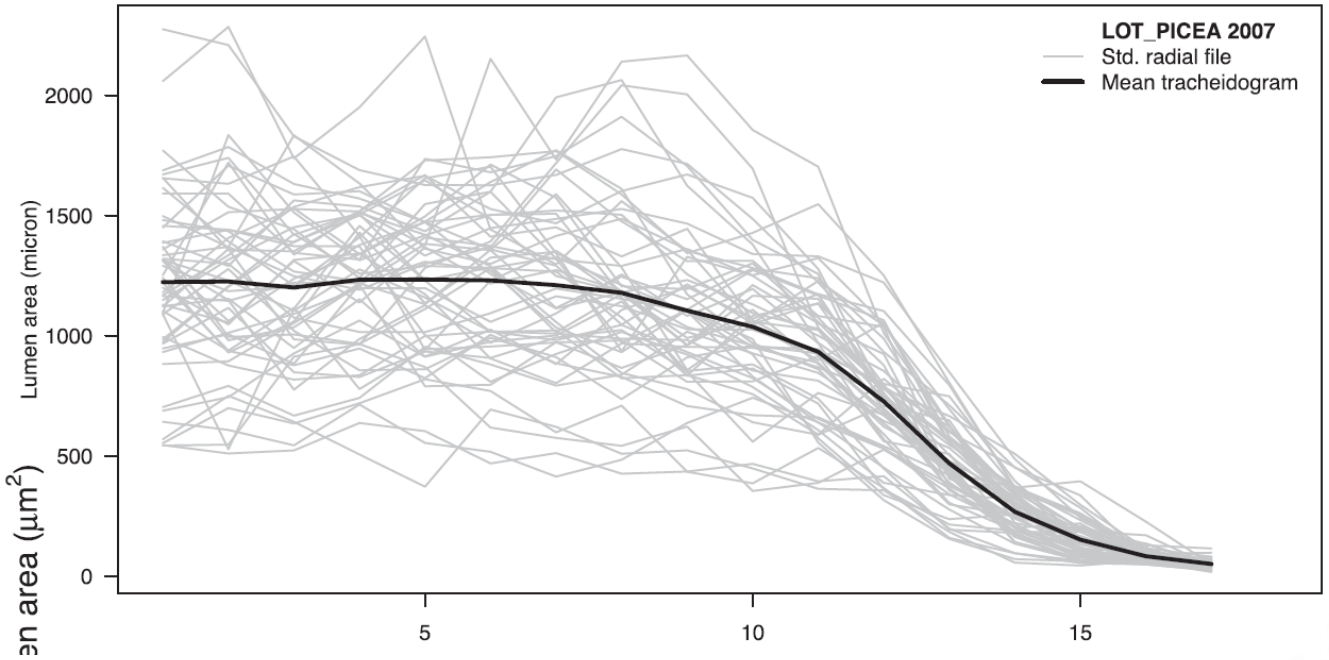
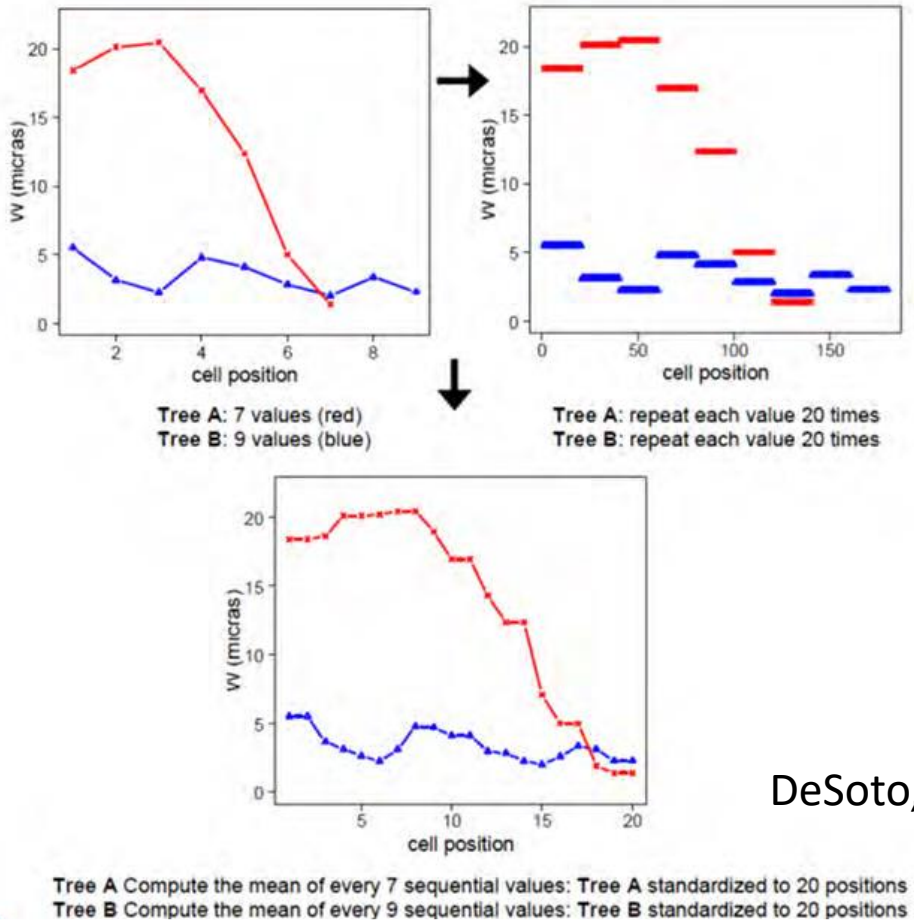


Figure 5. Data normalization procedure as described by Vaganov (1990) y Vaganov et al. (2006) to get the same number of cells in radial files (normalized tracheidogram, i.e. a curve showing variations in cell parameters as a function of the cell position within an annual ring, for instance, 20 cells per radial file in the figures).

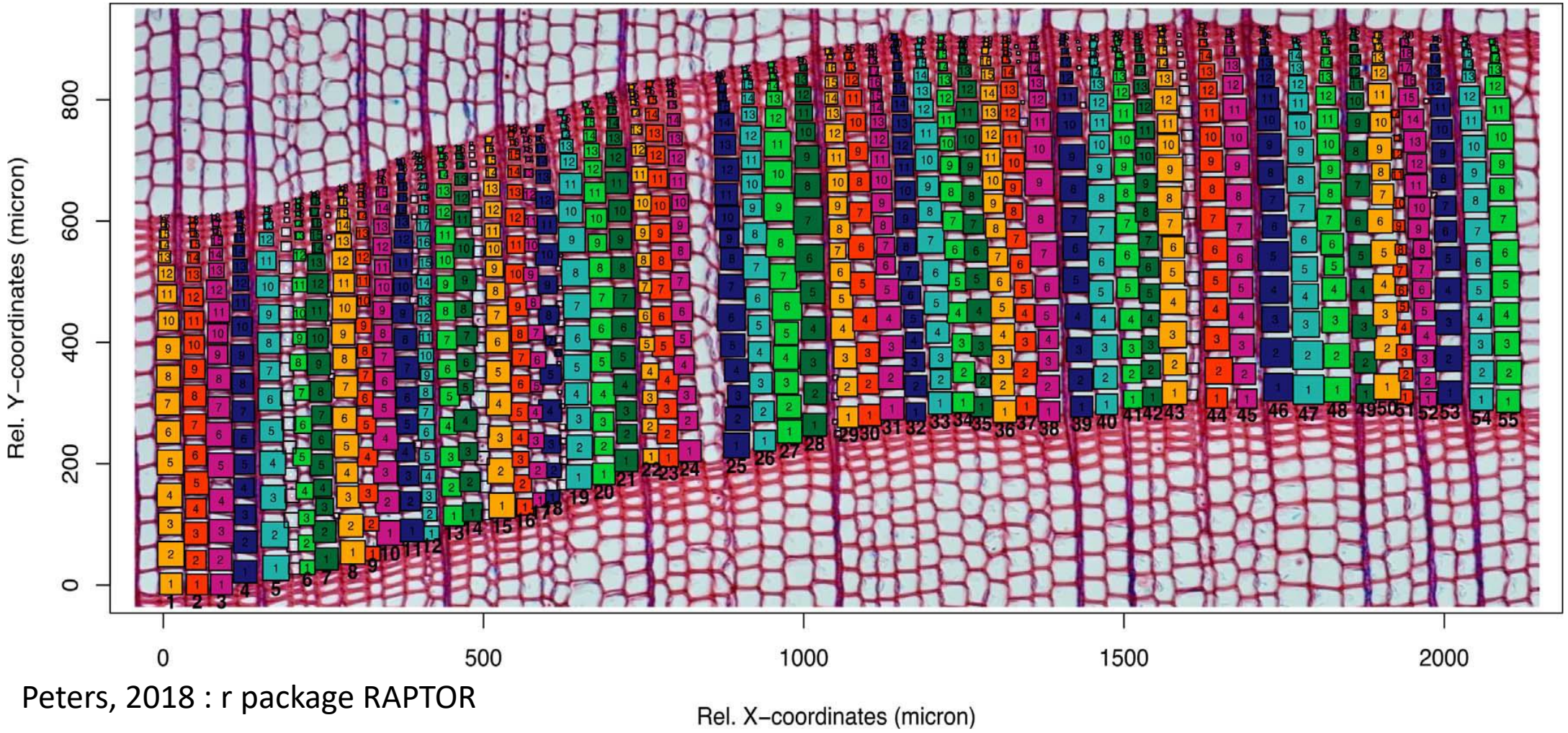


DeSoto, 2011

Quantitative Wood Anatomy

Data analysis methods

Tree-ring sectorization & Tracheidograms

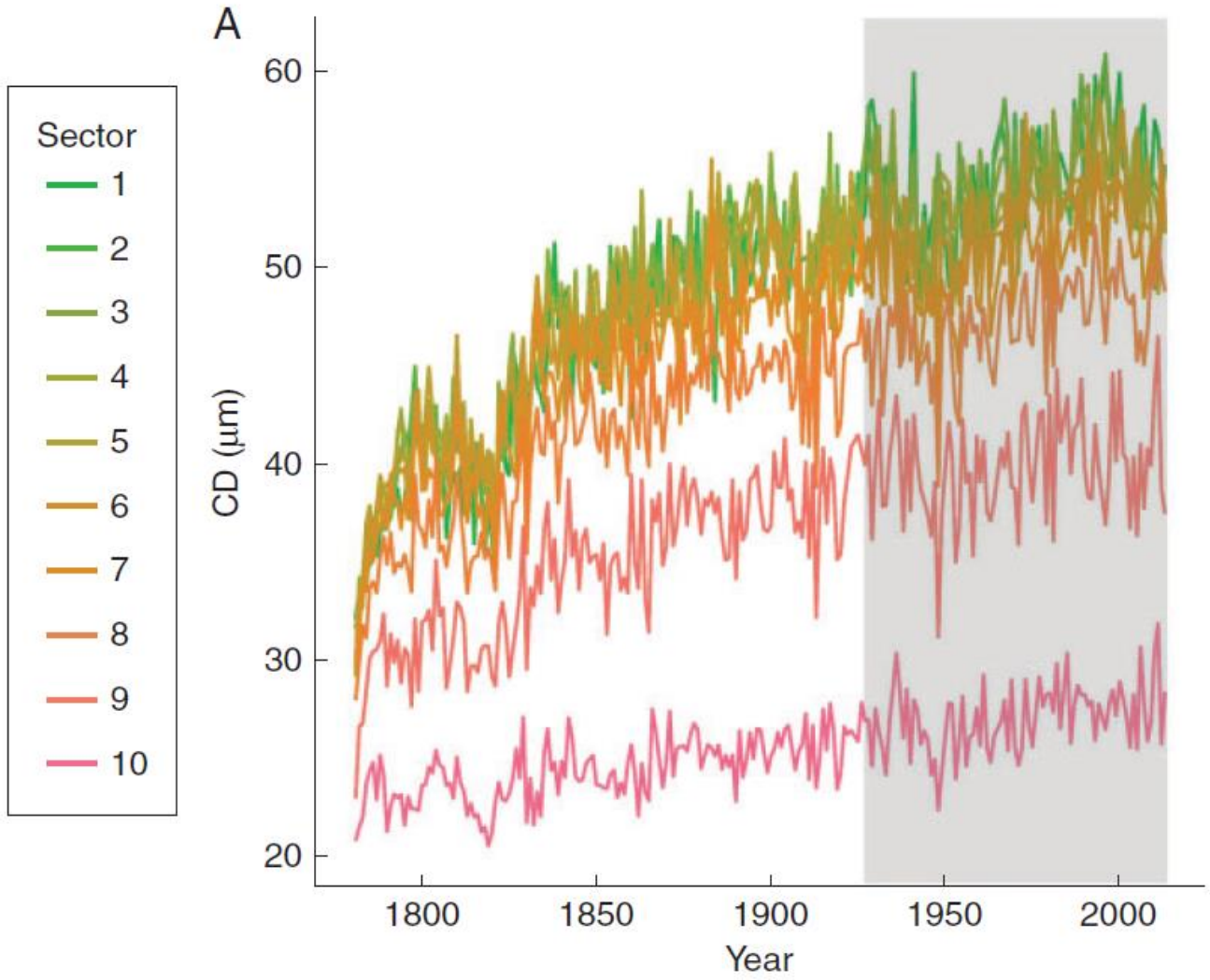


Peters, 2018 : r package RAPTOR

Quantitative Wood Anatomy

Data analysis methods

Tree-ring sectorization & Tracheidograms :
Conduit diameter Age trend

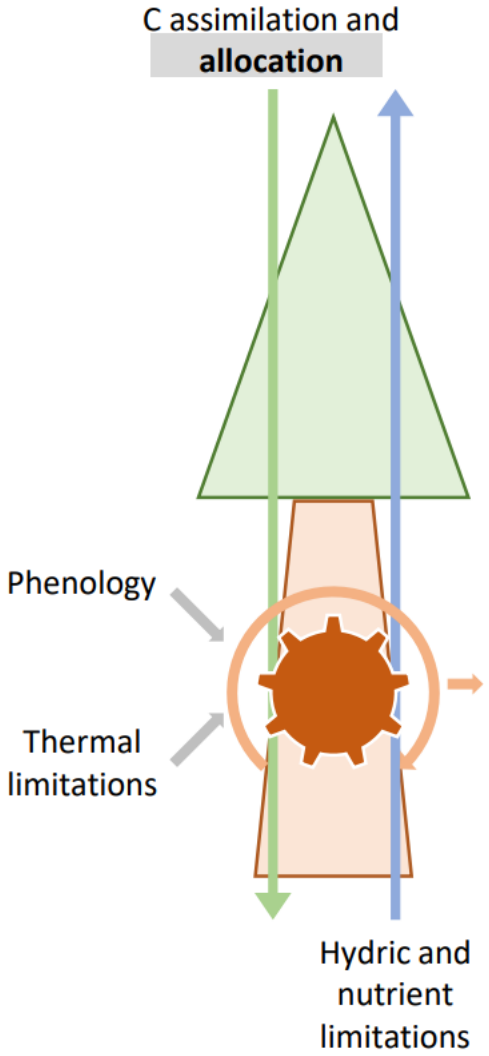


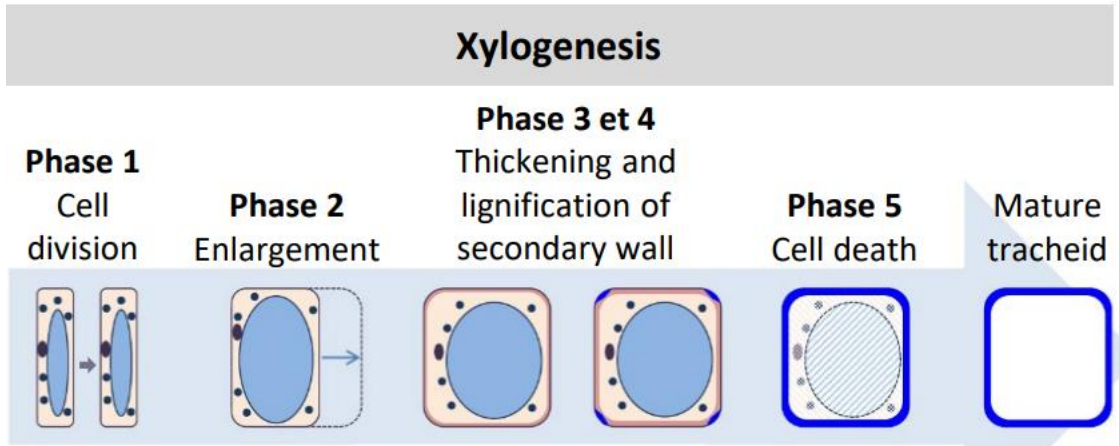
Castagneri, 2017

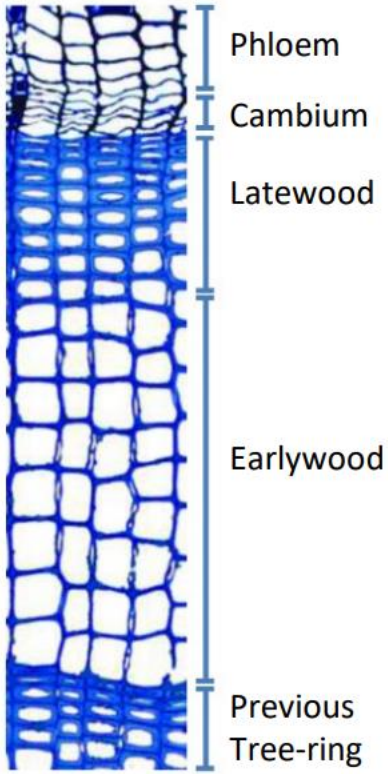
Quantitative Wood Anatomy

Data analysis methods

Retrospective analysis & xylogenesis







Cell Number, Cell Size and Cell Wall Thickness reflect differences in rate and duration of the successive phases of xylogenesis during the growing season

F.Gennaretti, modified from Cuny, 2014

Quantitative Wood Anatomy

Data analysis methods

Retrospective analysis & xylogenesis

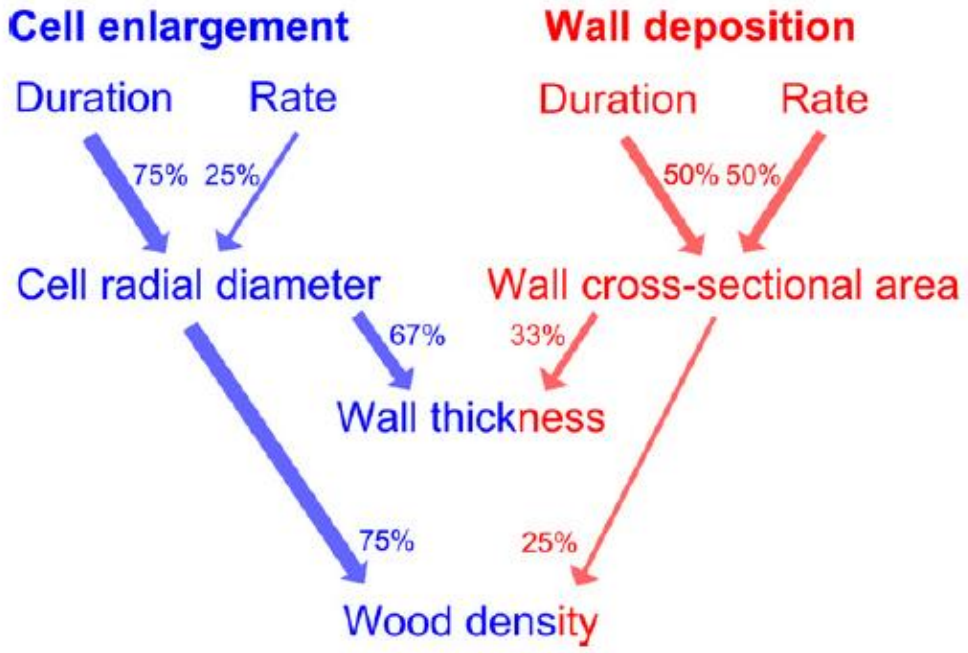
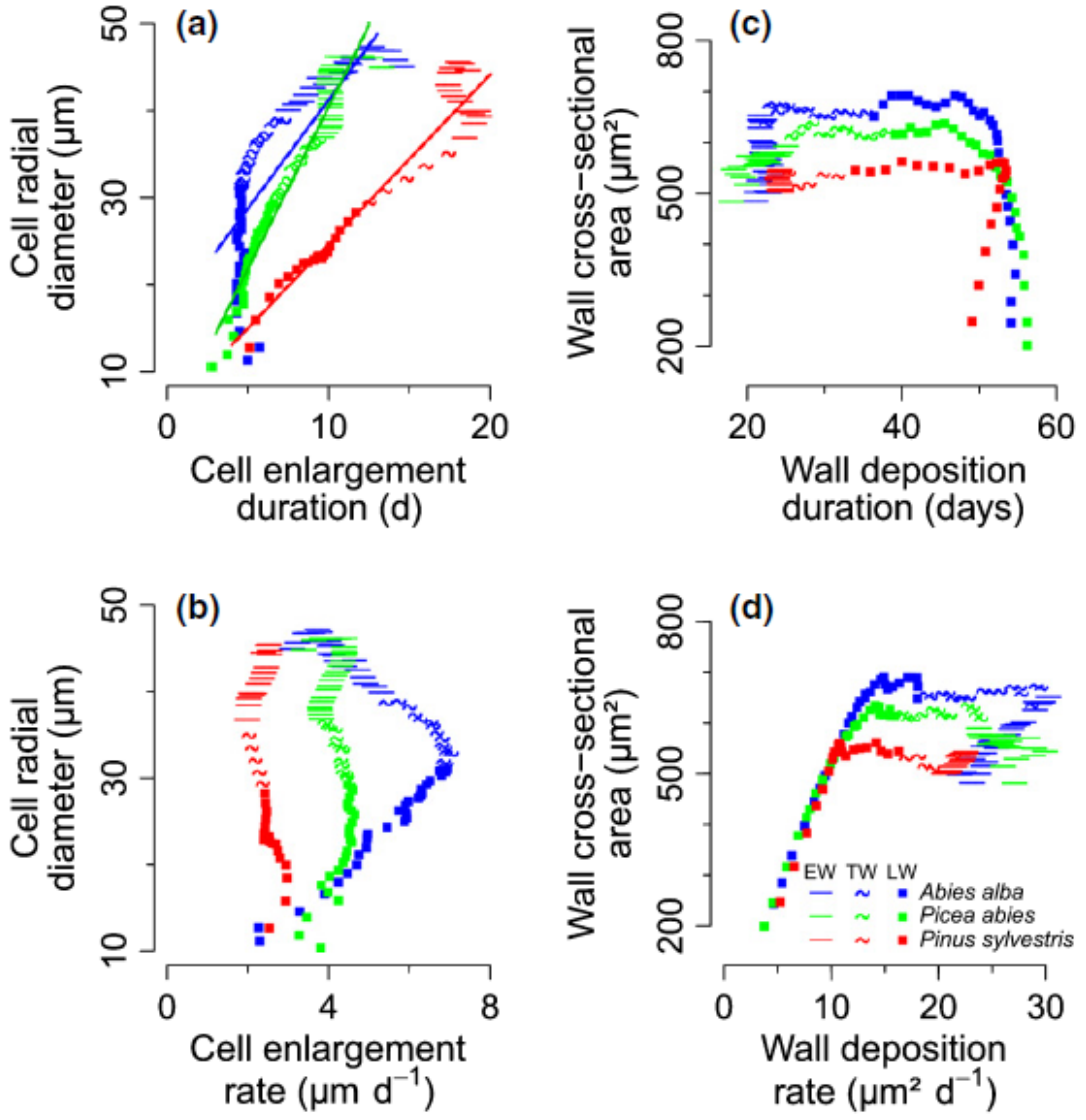


Fig. 8 Relative contributions of the different components of the kinetics of tracheid development to the changes in tracheid dimensions and wood density along the ring. The percentages provided were calculated on the basis of sensitivity analyses.

Cuny, 2014



Quantitative Wood Anatomy

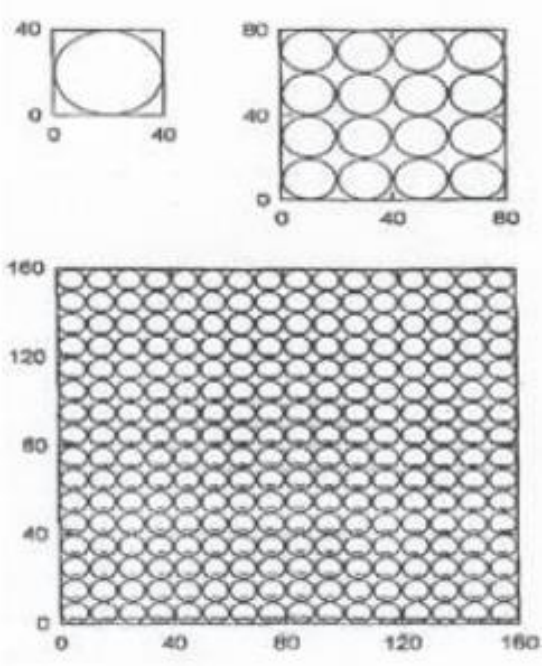
Wood anatomical traits as indicators of functional properties

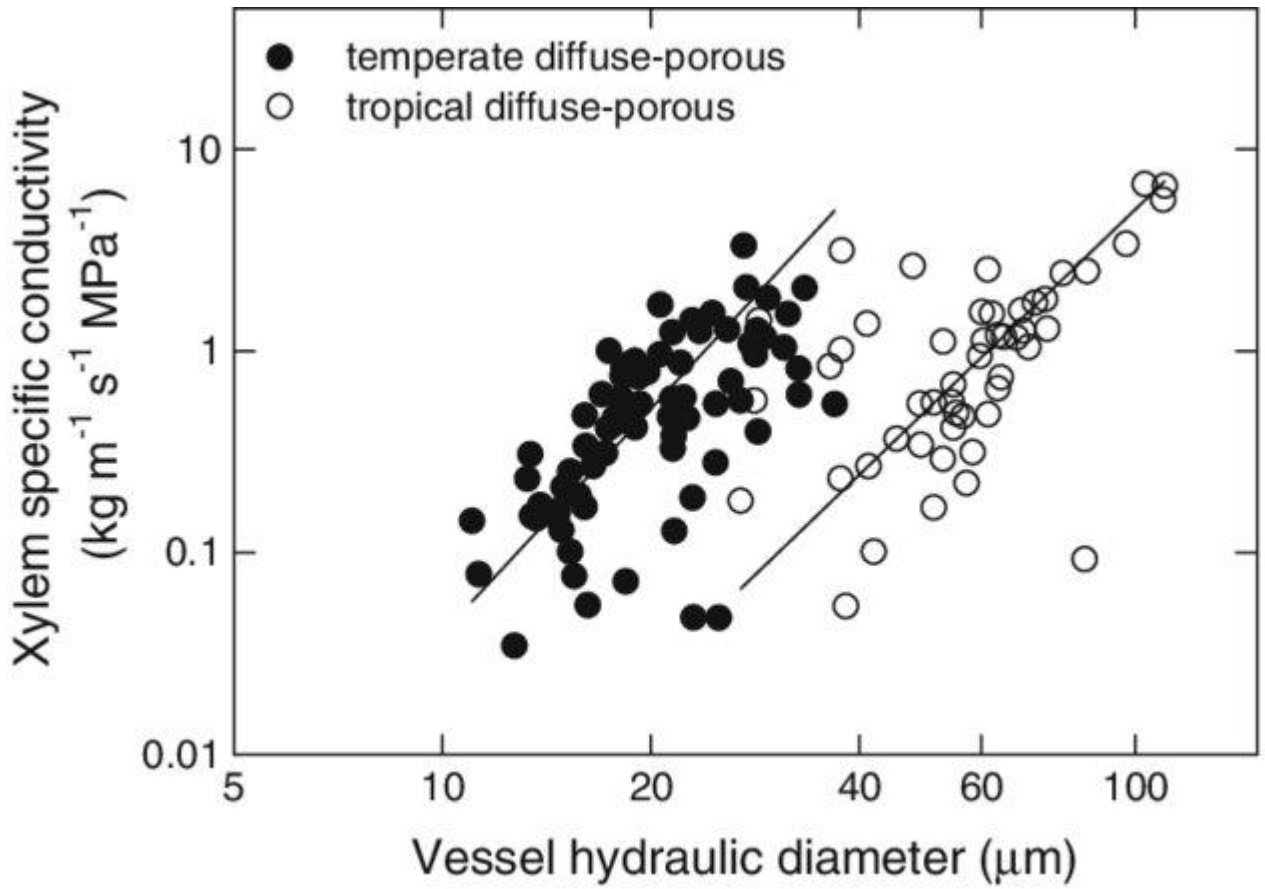
Relationships between anatomy & hydraulic properties

Safety vs. **Efficiency**

Meinzer & McCullough, 2010

Loi de Hagen-Poiseuille :
Le flux traversant un élément conducteur de diamètre d est identique à celui parcourant 16 éléments de diamètre égal à d/2 ou 256 éléments de diamètre égal à d/4. (D'après Tyree *et al.* 1994.)





Quantitative Wood Anatomy

Wood anatomical traits as indicators of functional properties

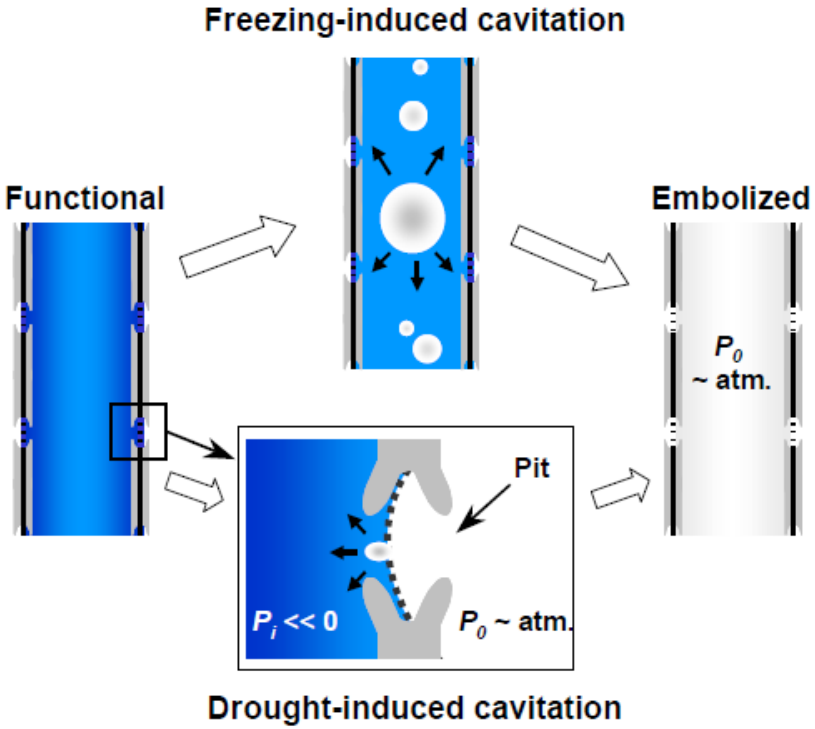
Relationships between anatomy & hydraulic properties

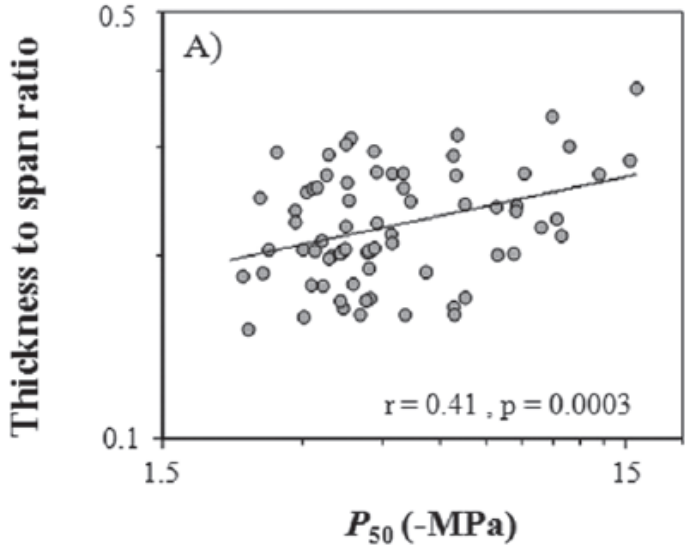
Safety vs. Efficiency

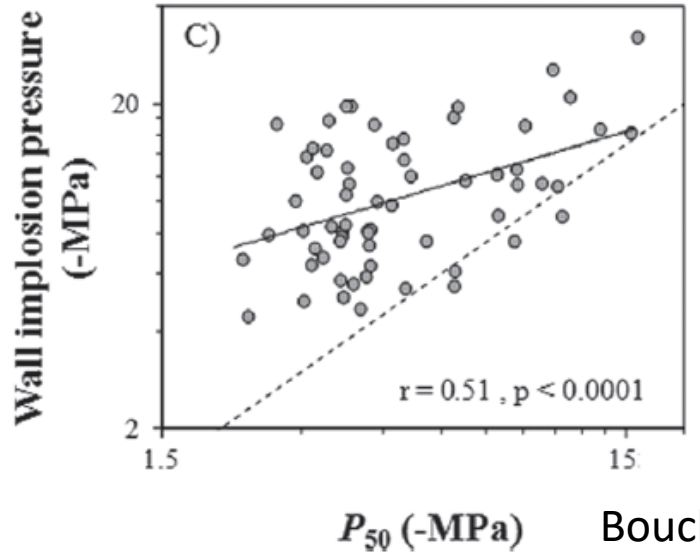
Hydraulic safety traits :

→ Larger conduits are more prone to embolism

→ Thicker walls seems to increase resistance to implosion





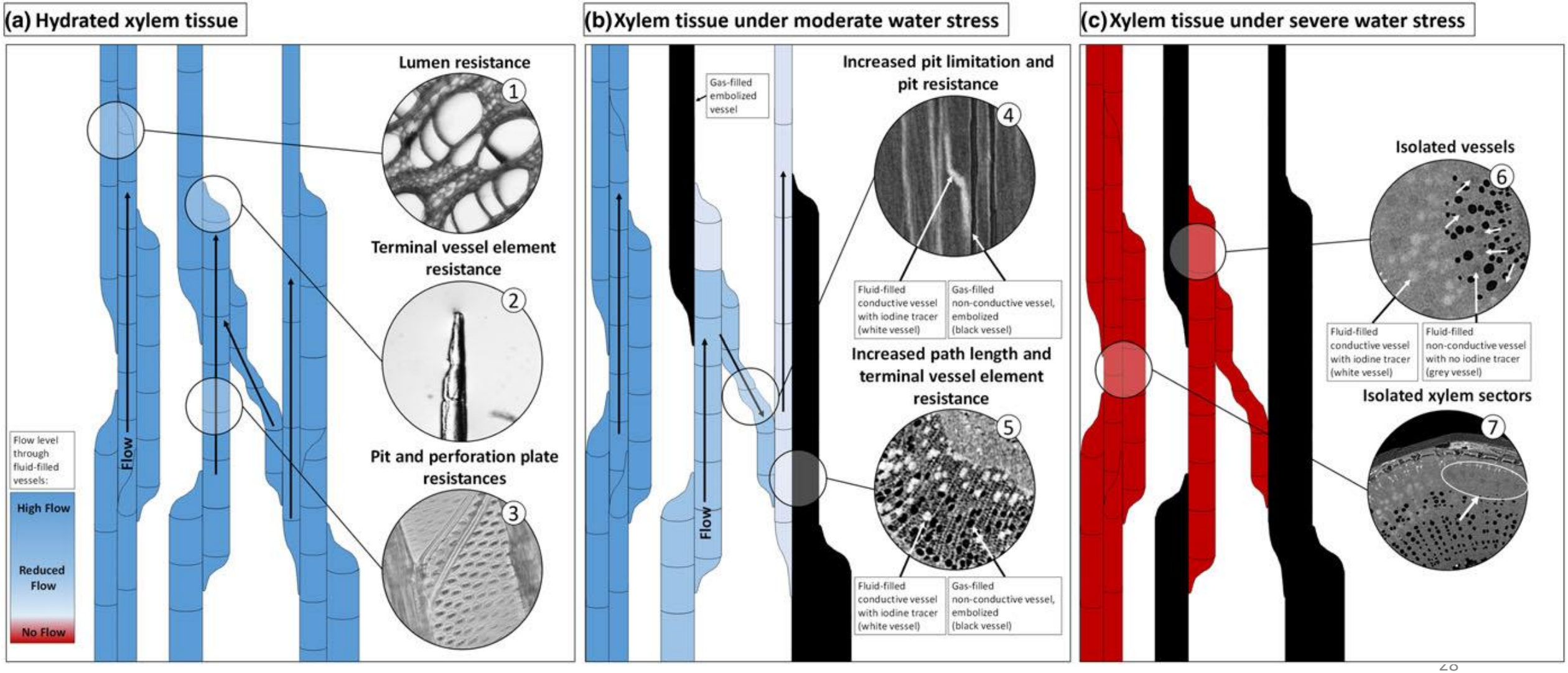


Bouche, 2014

Quantitative Wood Anatomy

Wood anatomical traits as indicators of functional properties

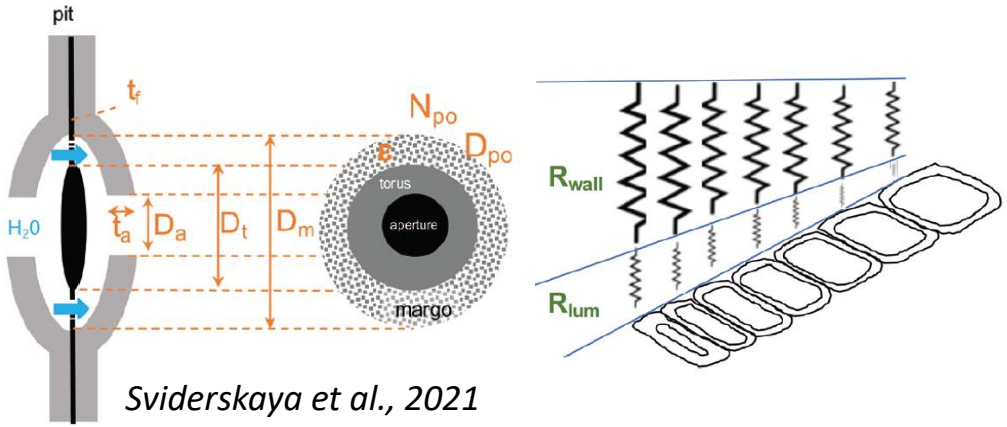
Relationships between anatomy & hydraulic properties



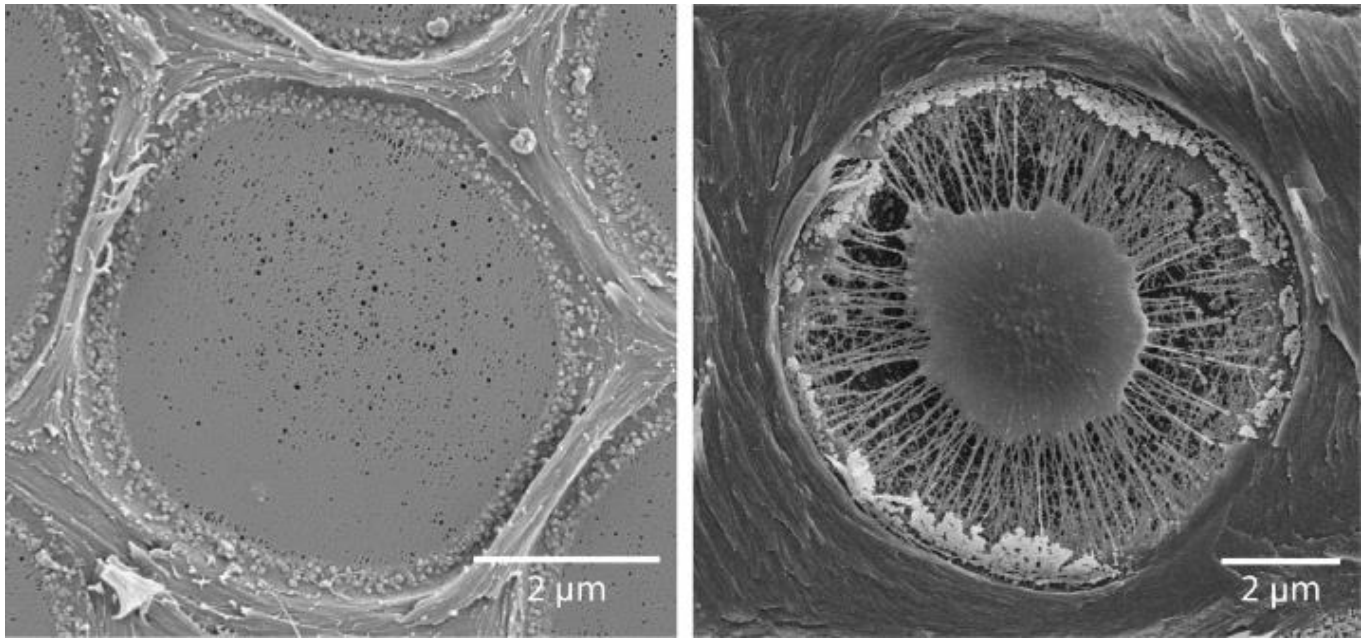
Quantitative Wood Anatomy

Pit influence on hydraulic efficiency and safety

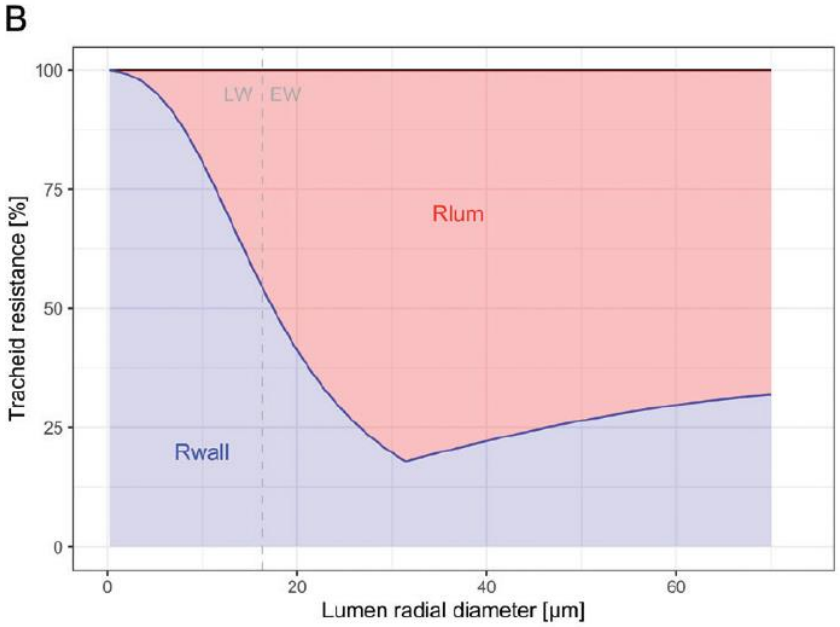
Pitterman et al., 2006 ; Hacke et al., 2004, 2006 ; Domec et al., 2006 ; Schulte et al., 2015 ; Bouche et al., 2014 ; Song et al., 2022 ; Sperry et al., 2006 ; Lens et al., 2011



Sviderskaya et al., 2021



Choat et al., 2009



- Relative contributions of Tracheid dimensions & Pit structure to hydraulic conductance & vulnerability to cavitation are not resolved
- Pit scale adjustements can be an efficient strategy to cope with drought
- Possible isometric scaling to take into account pit variability in estimations of hydraulic efficiency & safety ?

Quantitative Wood Anatomy

Wood anatomical traits as indicators of functional properties

Cuny, 2014

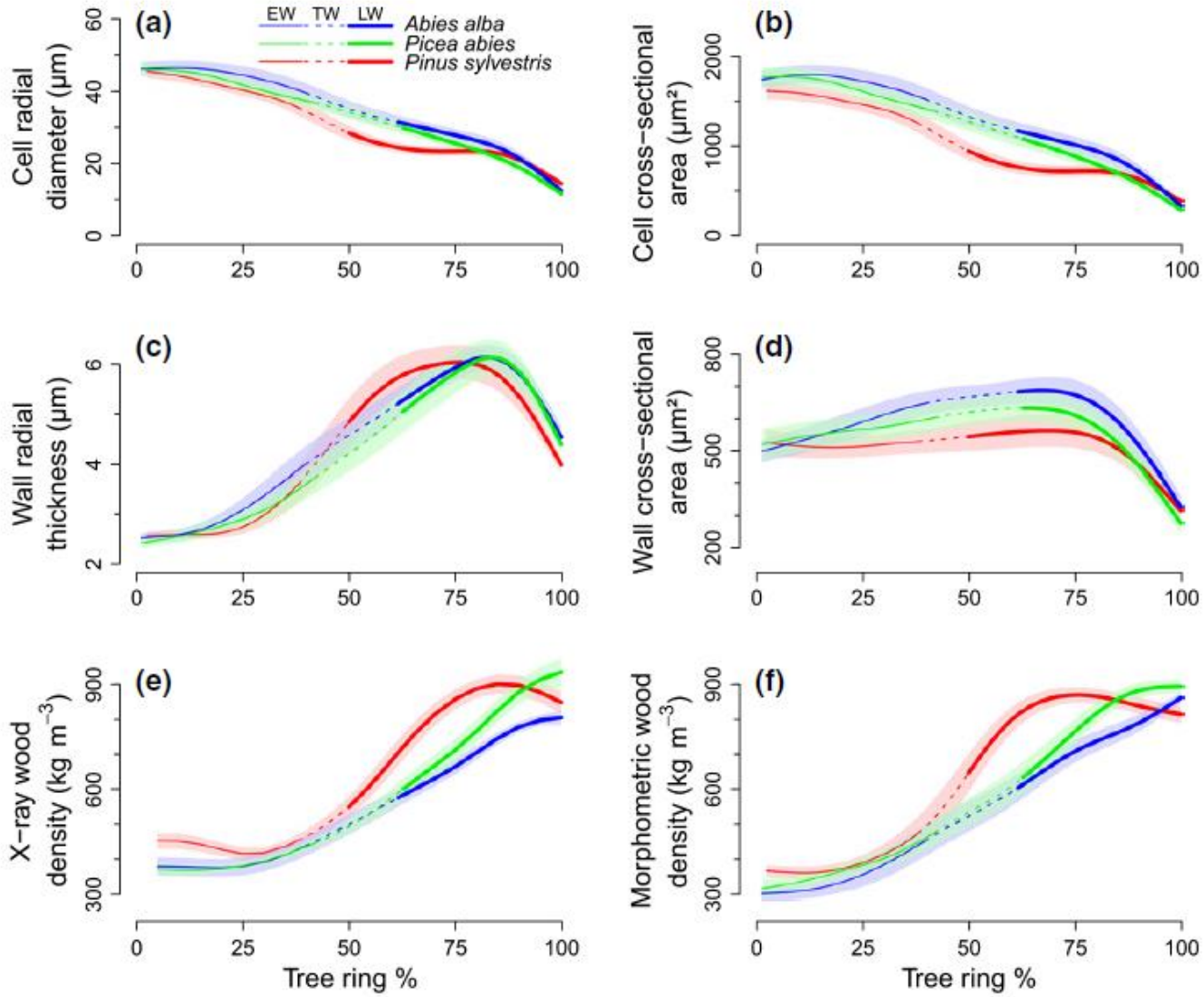
Relationships between Anatomy & Carbon Sequestration

Proxies of Carbon contained in tree-ring :

→ Morphometric density,

→ Anatomical density,

→ Tissue percentages



Quantitative Wood Anatomy

Application example : Climate reconstruction

Article

Fennoscandian tree-ring anatomy shows a warmer modern than medieval climate

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Received: 11 February 2023
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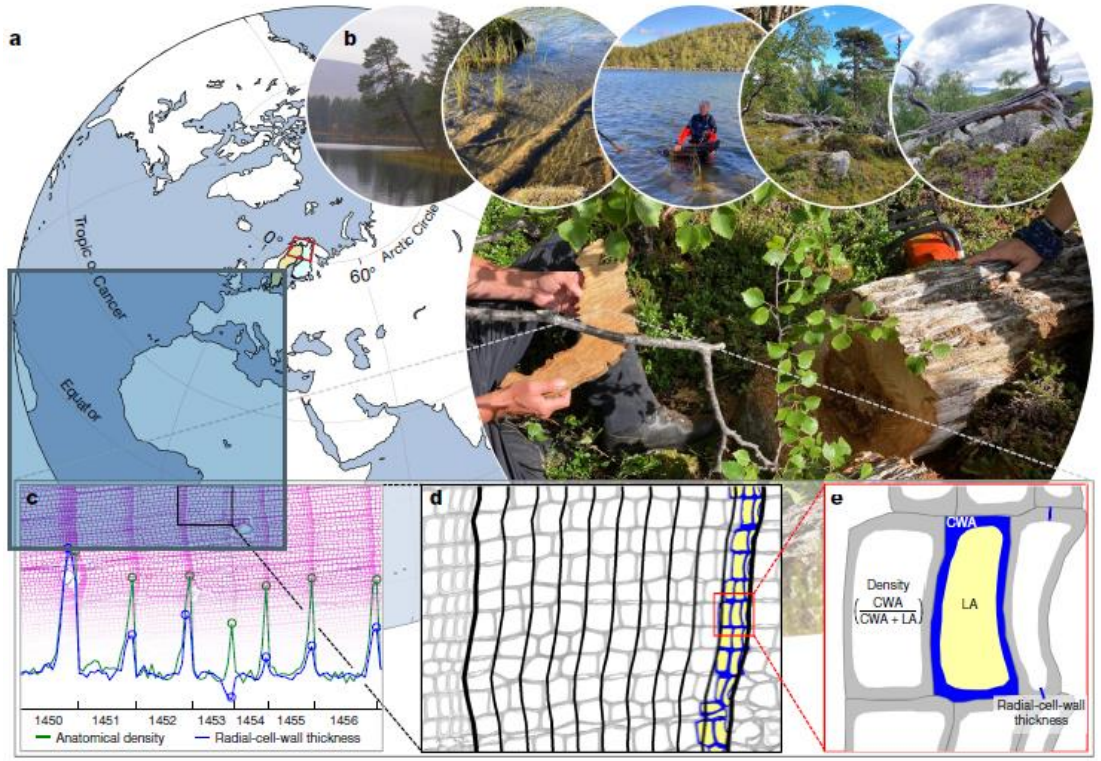
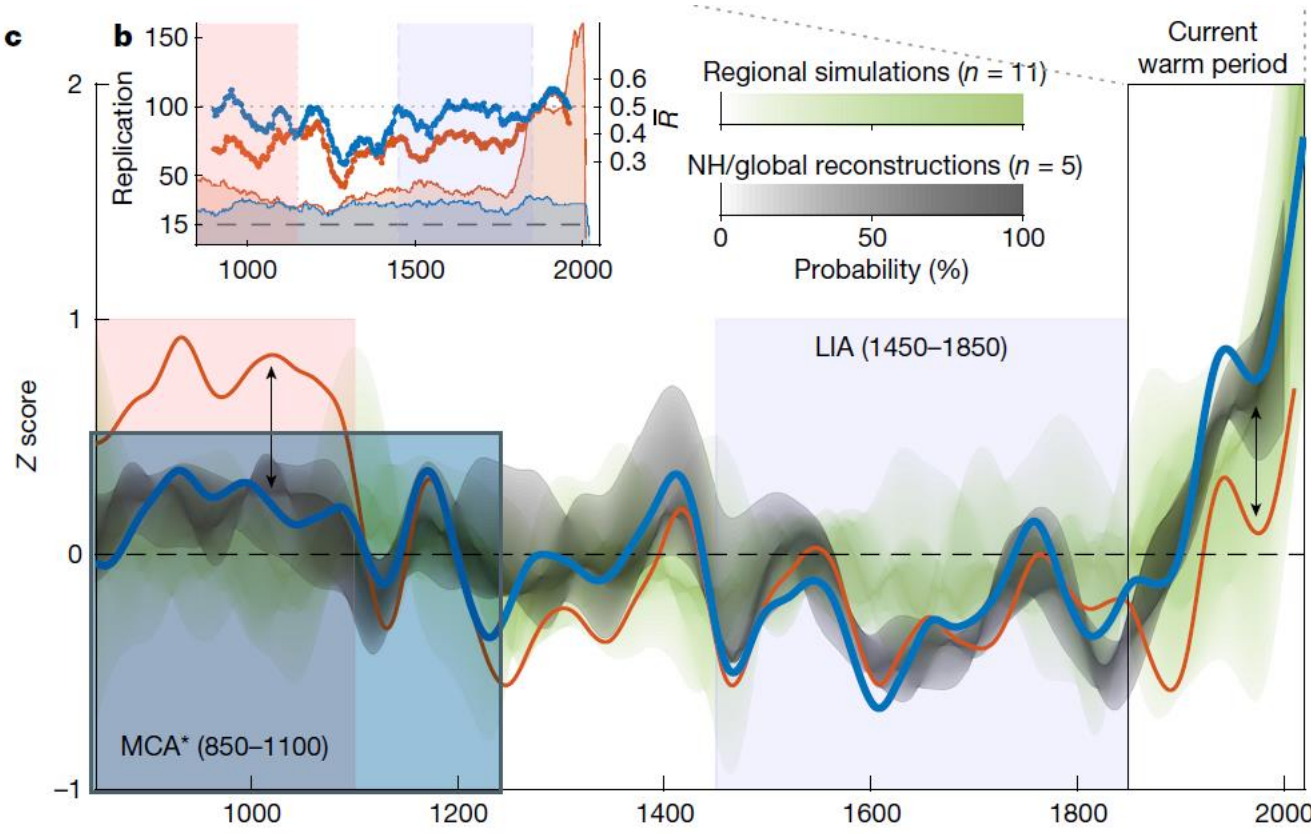
Published online: 2 August 2023

Jesper Björklund[1,2]✉, Kristina Seftigen[1,3], Markus Stoffel[4,5,6], Marina V. Fonti[1,2], Sven Kottlow[1], David C. Frank[7], Jan Esper[8,9], Patrick Fonti[1,2], Hugues Goosse[10], Håkan Grudd[11], Björn E. Gunnarson[12,13], Daniel Nievergelt[1,2], Elena Pellizzari[14], Marco Carrer[14,15] & Georg von Arx[1,2,15]

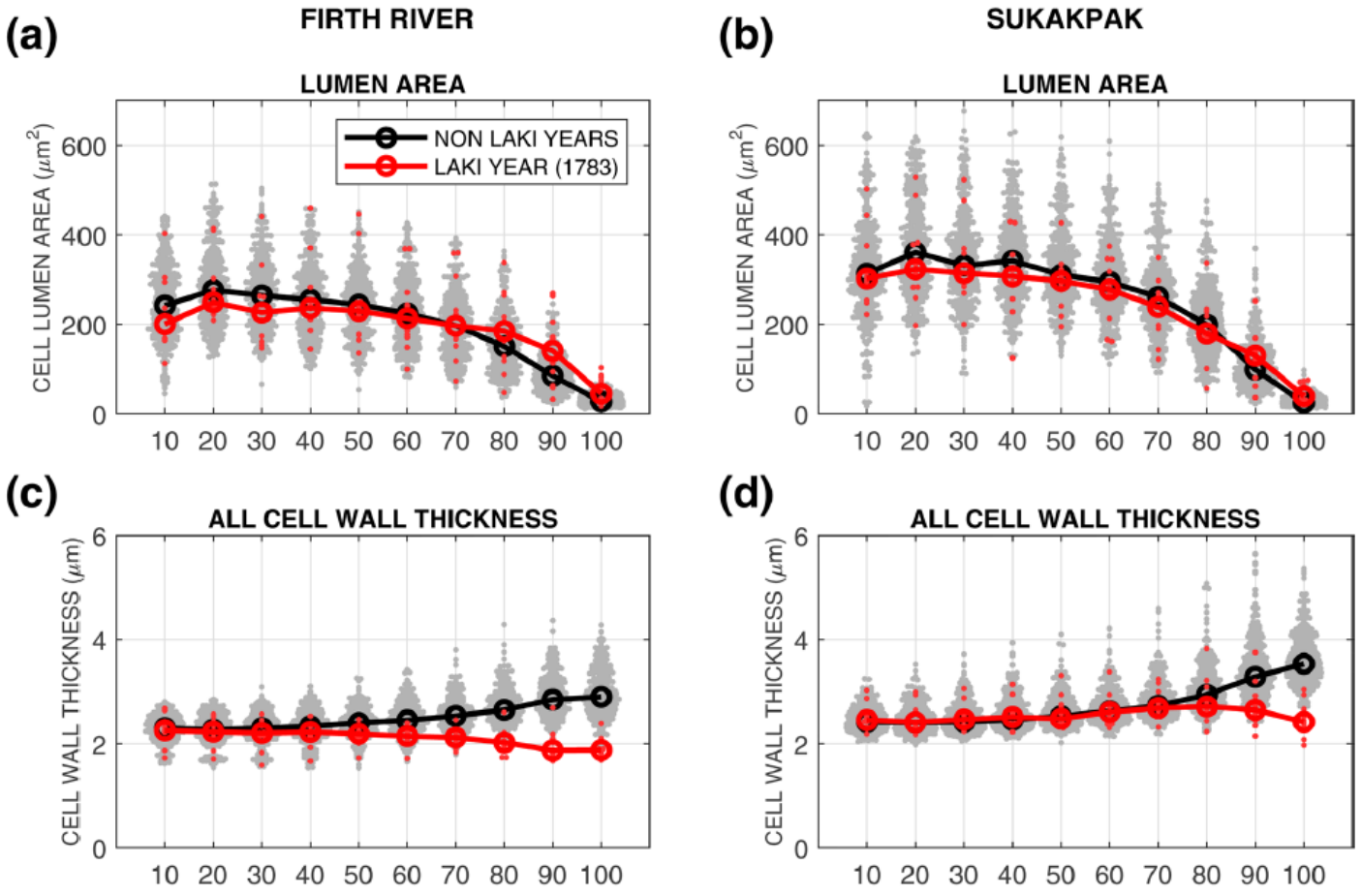


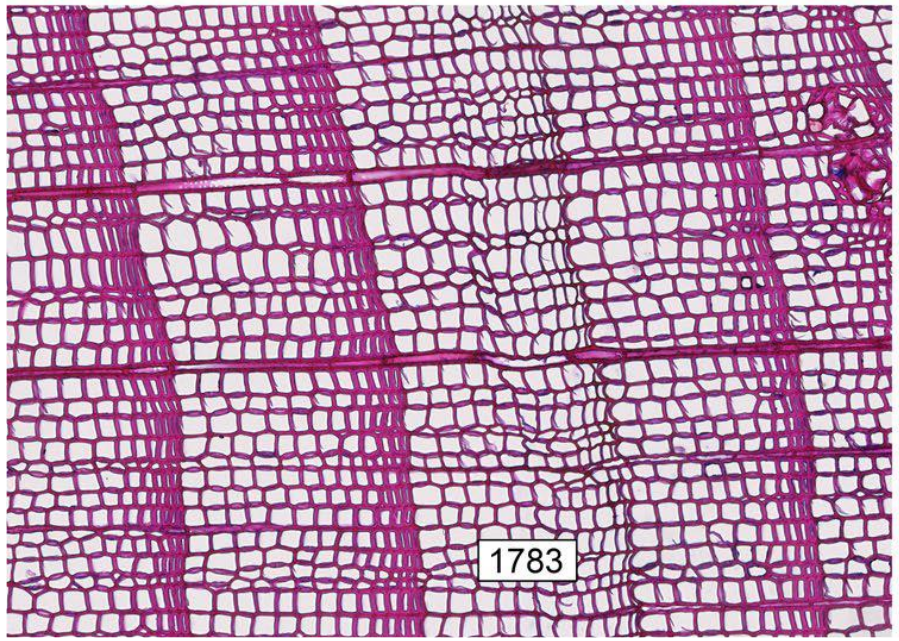


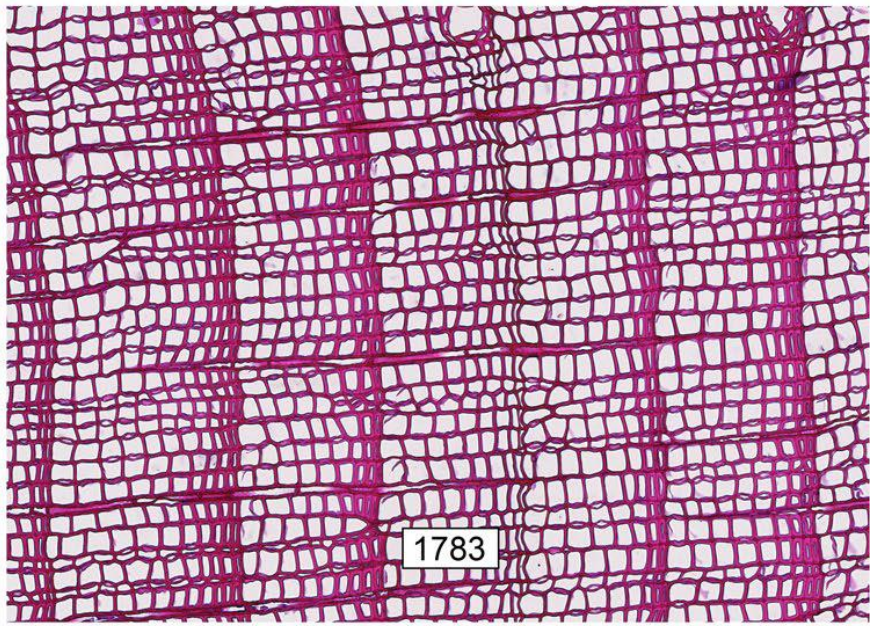
Quantitative Wood Anatomy

Application example : Volcanic eruption

Edwards, 2020 : Cell division & enlargement haven't been impacted
But abrupt and premature cessation of cell wall thickening
→ rapid temperature decrease toward the end of the growing season following the Laki eruption





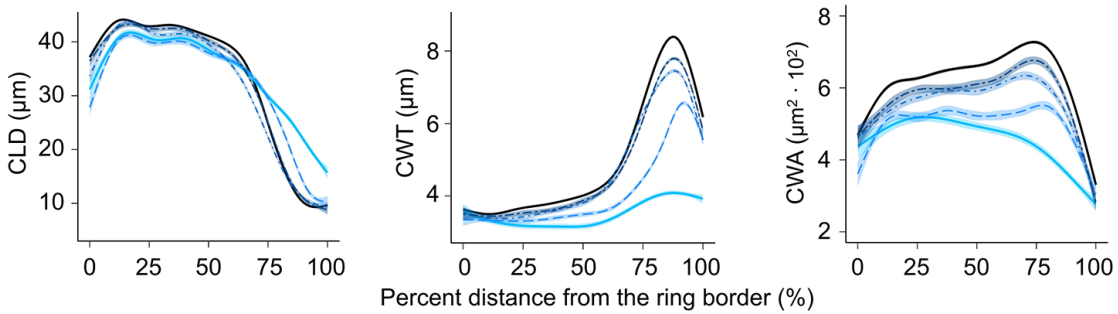


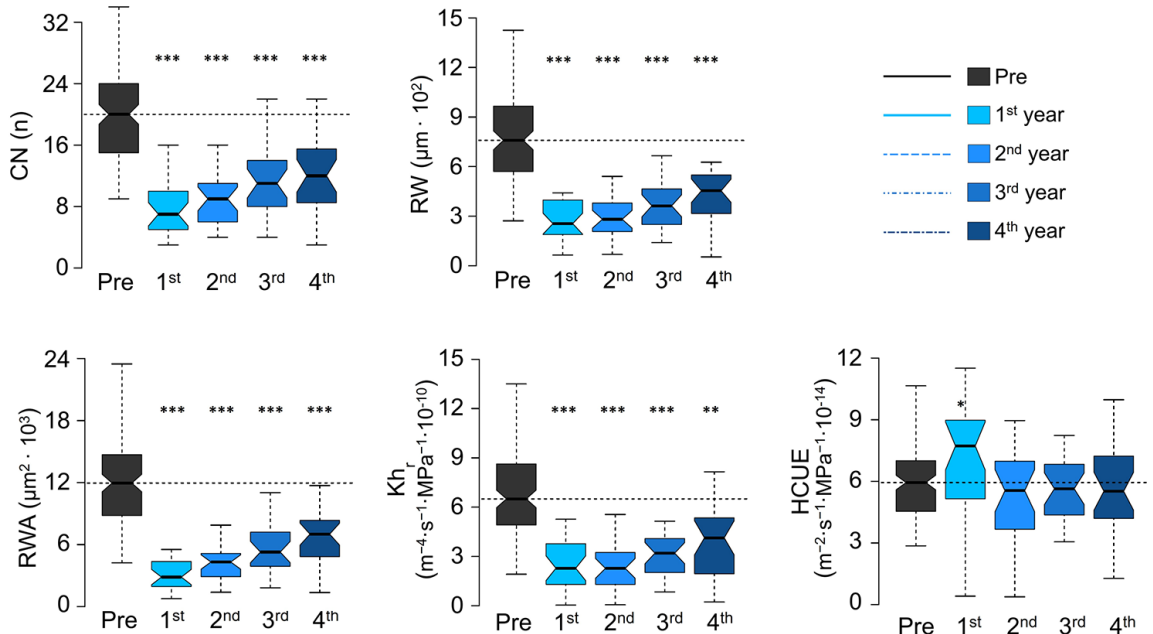
Quantitative Wood Anatomy

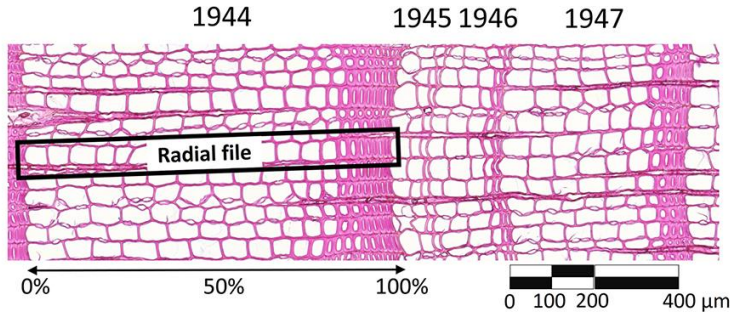
Application example : Insect outbreaks

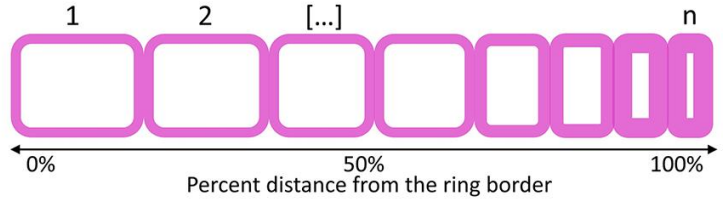
Long-Term Impacts of Defoliator Outbreaks on Larch Xylem Structure and Tree-Ring Biomass

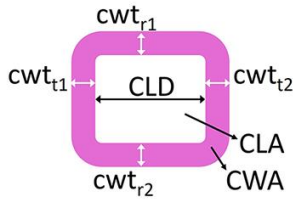
Daniele Castagneri[1], Angela L. Prendin[2], Richard L. Peters[1,3], Marco Carrer[2], Georg von Arx[1] and Patrick Fonti[1]*











Derived variables

$CWT = (cwt_{t1}+cwt_{t2}+cwt_{r1}+cwt_{r2})/4$

$CTA = CLA+CWA$

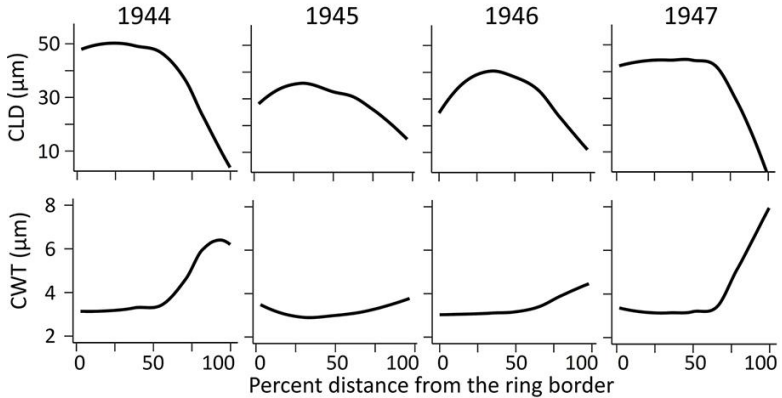
$CWD = CWA / (CWA+CLA)$

$Kh_c \sim CLA$

$Kh_r = \sum_n^1 Kh_c$

$RWA = \sum_n^1 CWA$

$HCUE = Kh_r / RWA$



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IAWA list of microscopic features for hardwood identification

IAWA list of microscopic features for softwood identification

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