

# **Lignin suppression in *Arabidopsis thaliana* mutant is unveiled by X-ray ptychography**

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**Brazilian Synchrotron Light Laboratory (LNLS)**

Cateretê group (coherent x-ray scattering imaging and small angle X-ray beamline)

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## **Coherent scattering applied to life sciences**

- X-ray ptychography for biological tissue study

## **X-ptychography: plant sciences**

- Biomass deconstruction: ethanol production
- Ptychography X-ray Computed Tomography (PXCT)
- Cellular compartments quantification

## **Conclusion remarks**

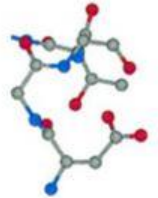
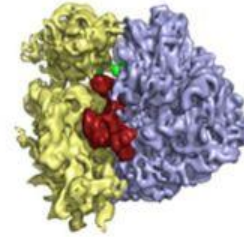
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**Organisms**

**Tissue**

**Cells**

**Organelles**

**Complexes**

**Proteins**

**Atoms**

**Computerised Tomography**

1-2 mm

**Fluorescence Microscopy**

250 nm

**Cryo-Soft X-ray Tomography**

25 nm

**Small Angle Scattering**

1 nm

**Cell Electron Tomography**

5 nm

**Single Particle Electron Microscopy**

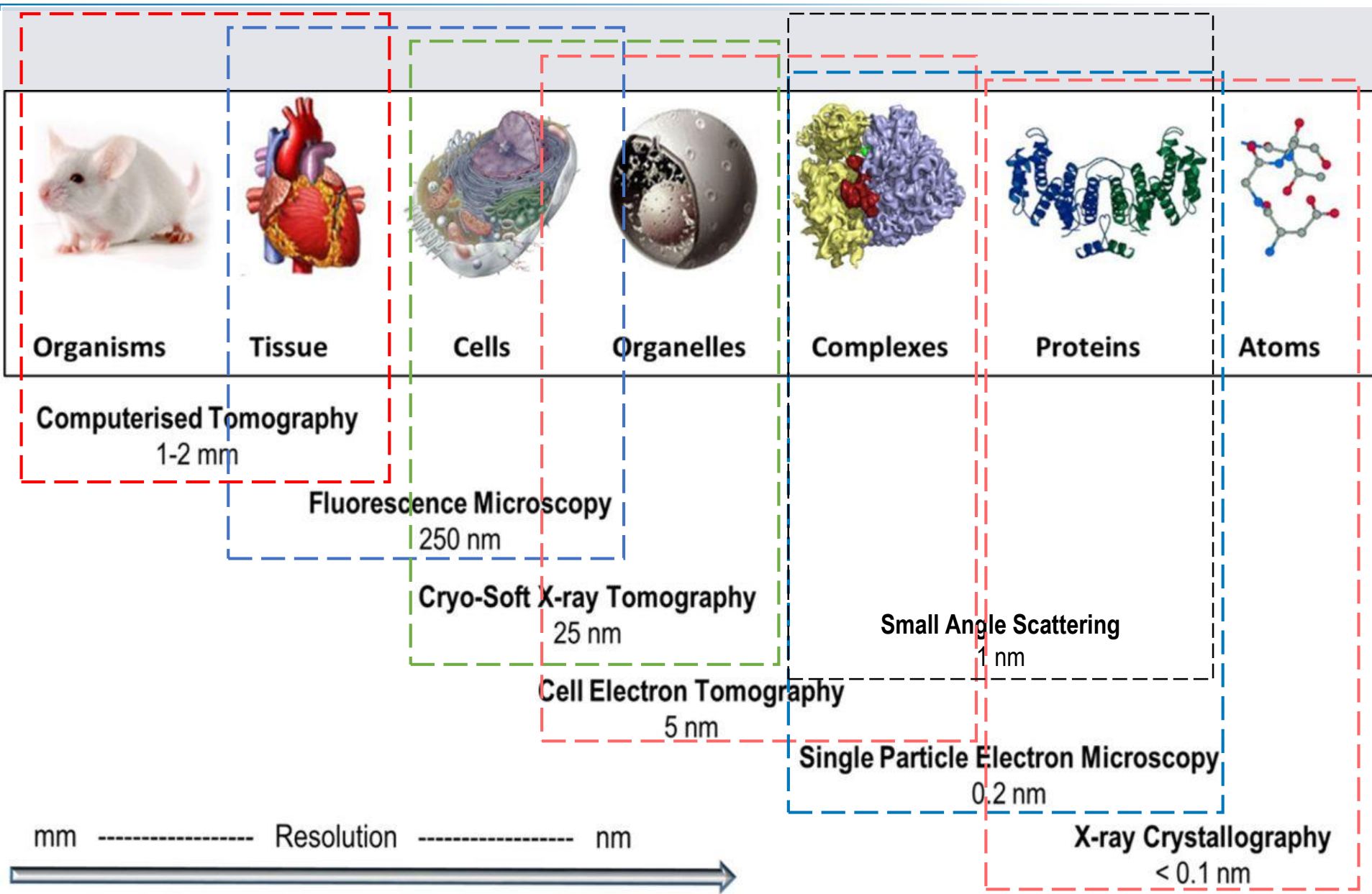
0.2 nm

**X-ray Crystallography**

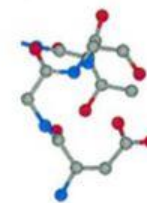
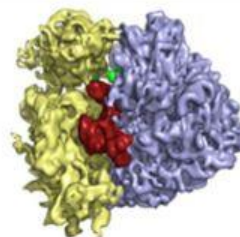
< 0.1 nm

mm ----- Resolution ----- nm





## Coherent X-ray imaging



**Organisms**

**Tissue**

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**Proteins**

**Atoms**

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1-2 mm

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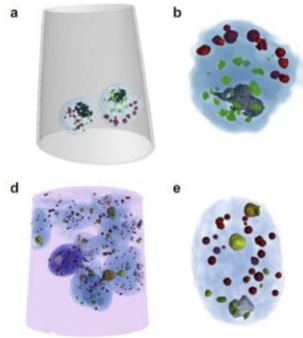
< 0.1 nm

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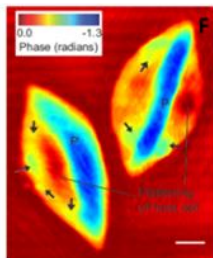


- Study of non-crystalline targets
- Experiments with specimens without chemical fixation
- Natural contrast to recover cellular and intracellular volume information



***C. reinhardtii* (algae)**

Diaz et al., *J Struc Biol*, 2015

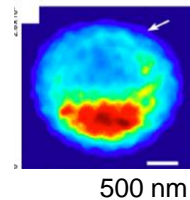


***F. falciparum* inside the host red blood cell (protozoan)**

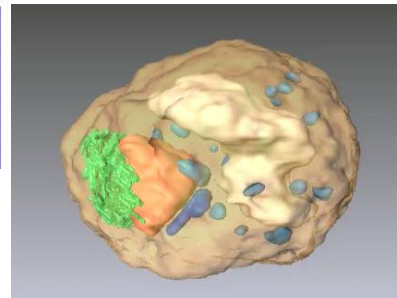


***Neospora caninum* (protozoan)**  
(plane-wave)

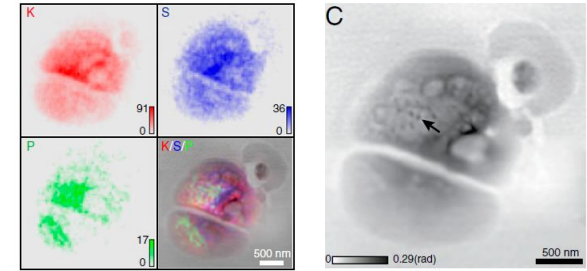
Rodriguez et al., *IUCr*, 2015



500 nm

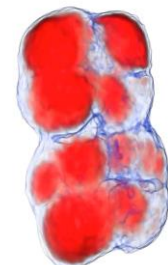
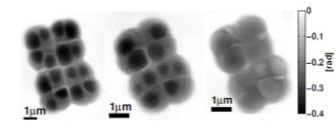


***S. pombe* spore (yeast)**



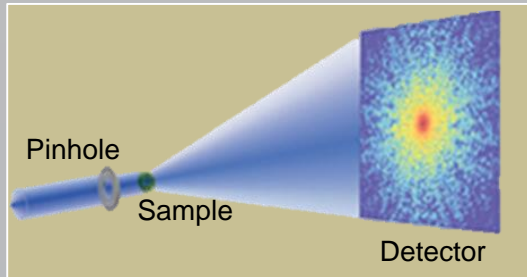
***Ostreococcus* sp. (algae)**

Deng et al., *PNAS*, 2015

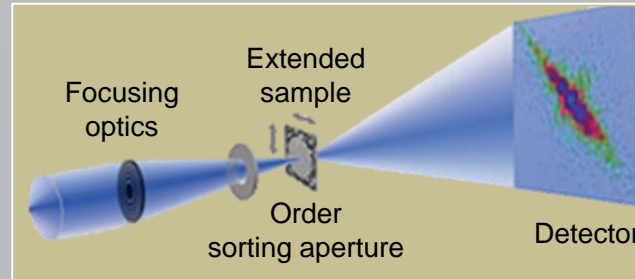


## Coherent X- Ray Imaging

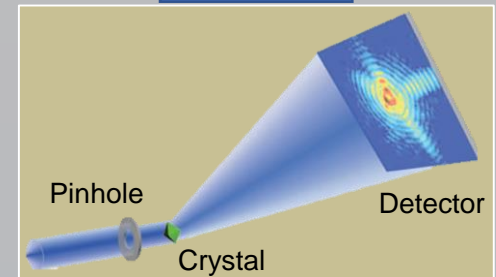
### Plane wave



### Ptychography



### Bragg

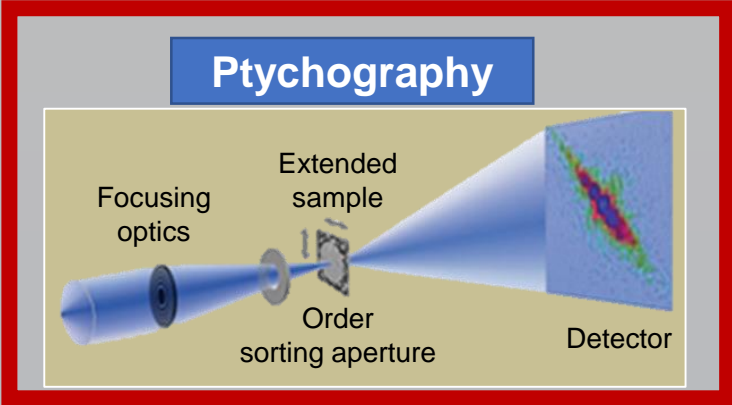
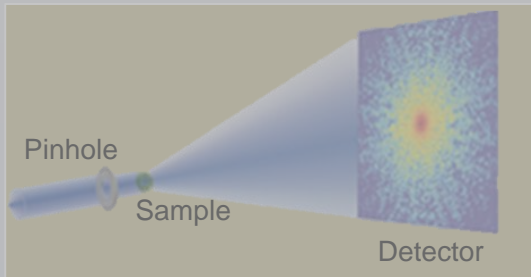


Miao J. et al., *Science*, Vol. 348, Issue 6234, pp. 530-535 (2015).

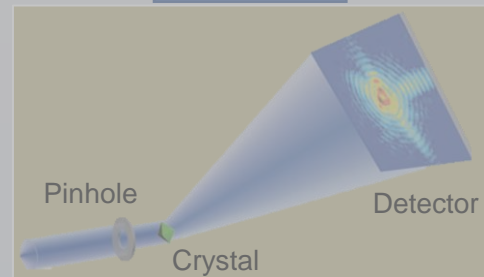


## Coherent X-Ray Imaging

### Plane wave

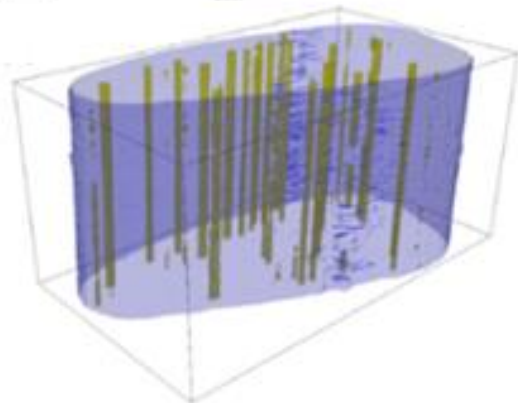
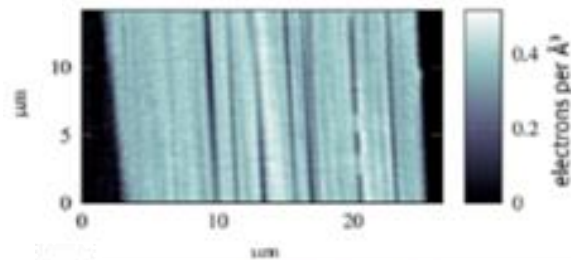


### Bragg



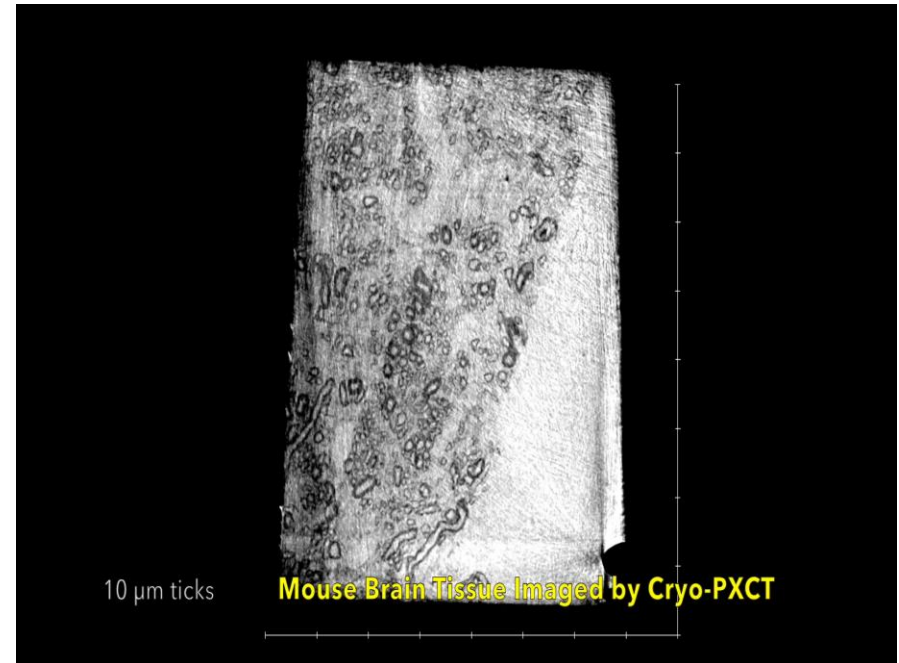
Miao J. et al., *Science*, Vol. 348, Issue 6234, pp. 530-535 (2015).

- Quantitative studies of soft tissue
- Porous network mapping
- Information recovery from the tens of micrometers size with few tens of nanometers resolution



**Silk fiber porosity**  
(ptychography)

*Esmaeili et al., Macromolecules 2013*



**Mouse brain tissue**  
(ptychography)

*Shahmoradian et al., Sci Rep, 2017*

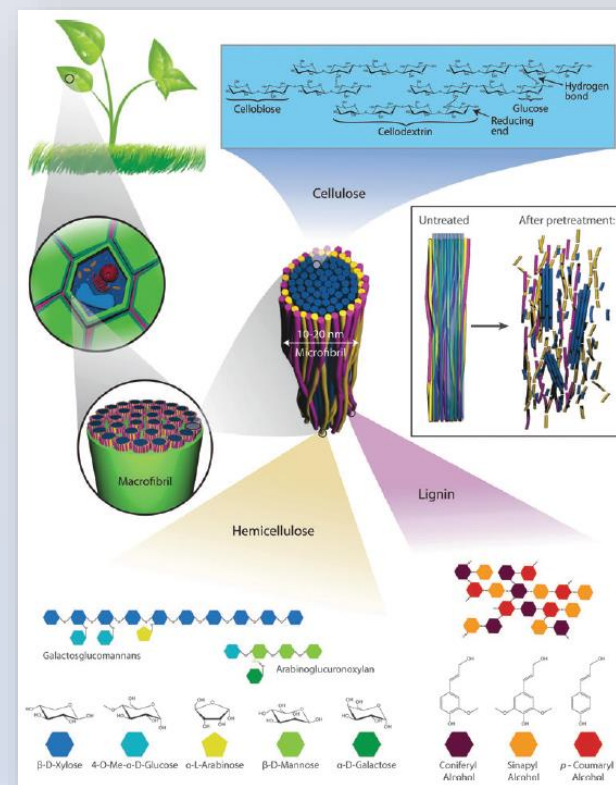
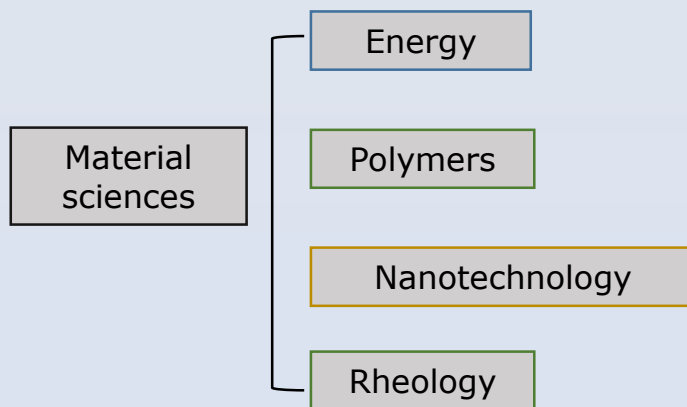
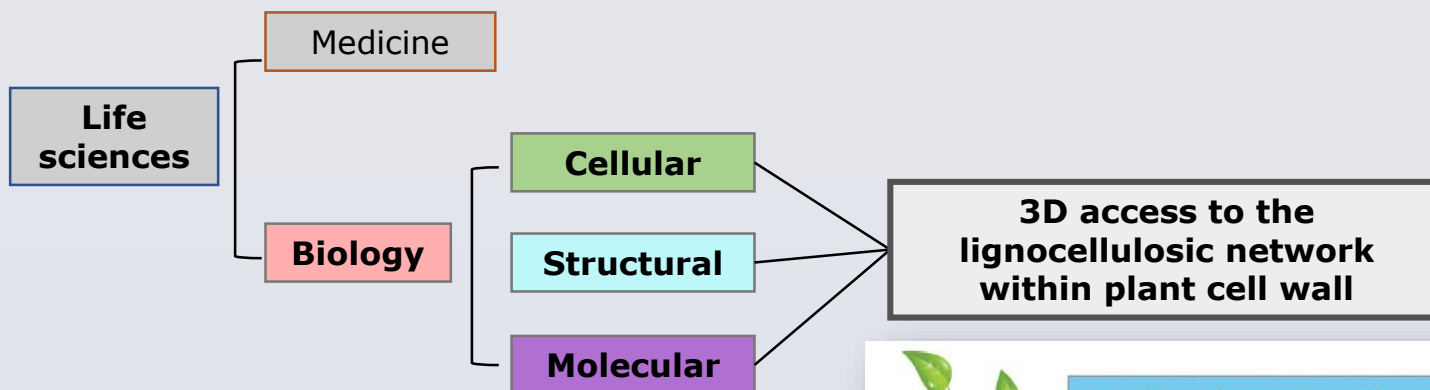
## Coherent scattering applied to life sciences

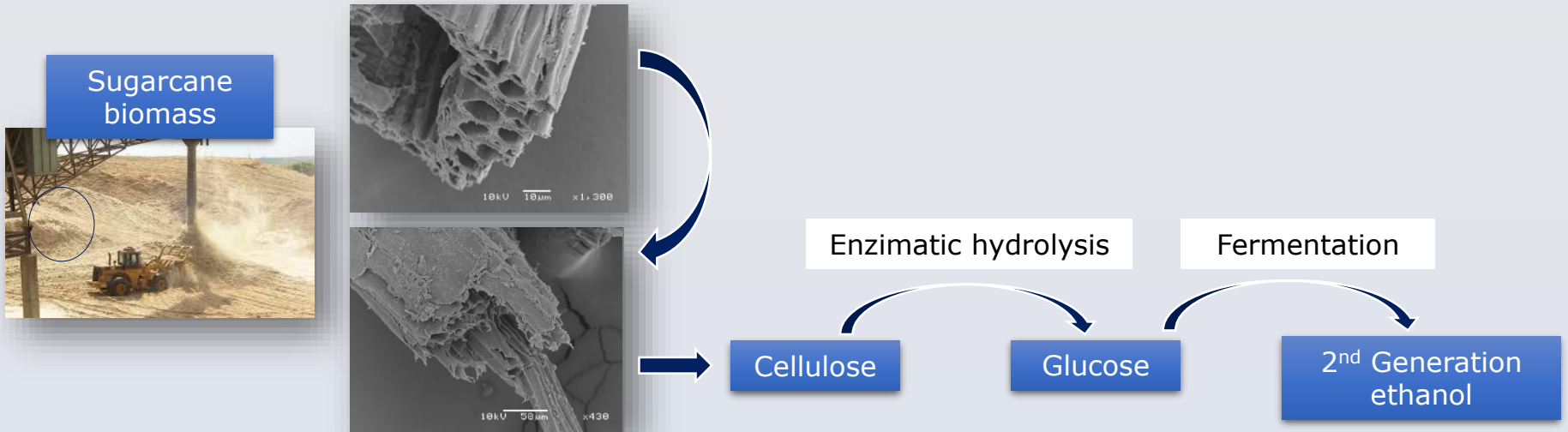
- X-ray ptychography for biological tissue study

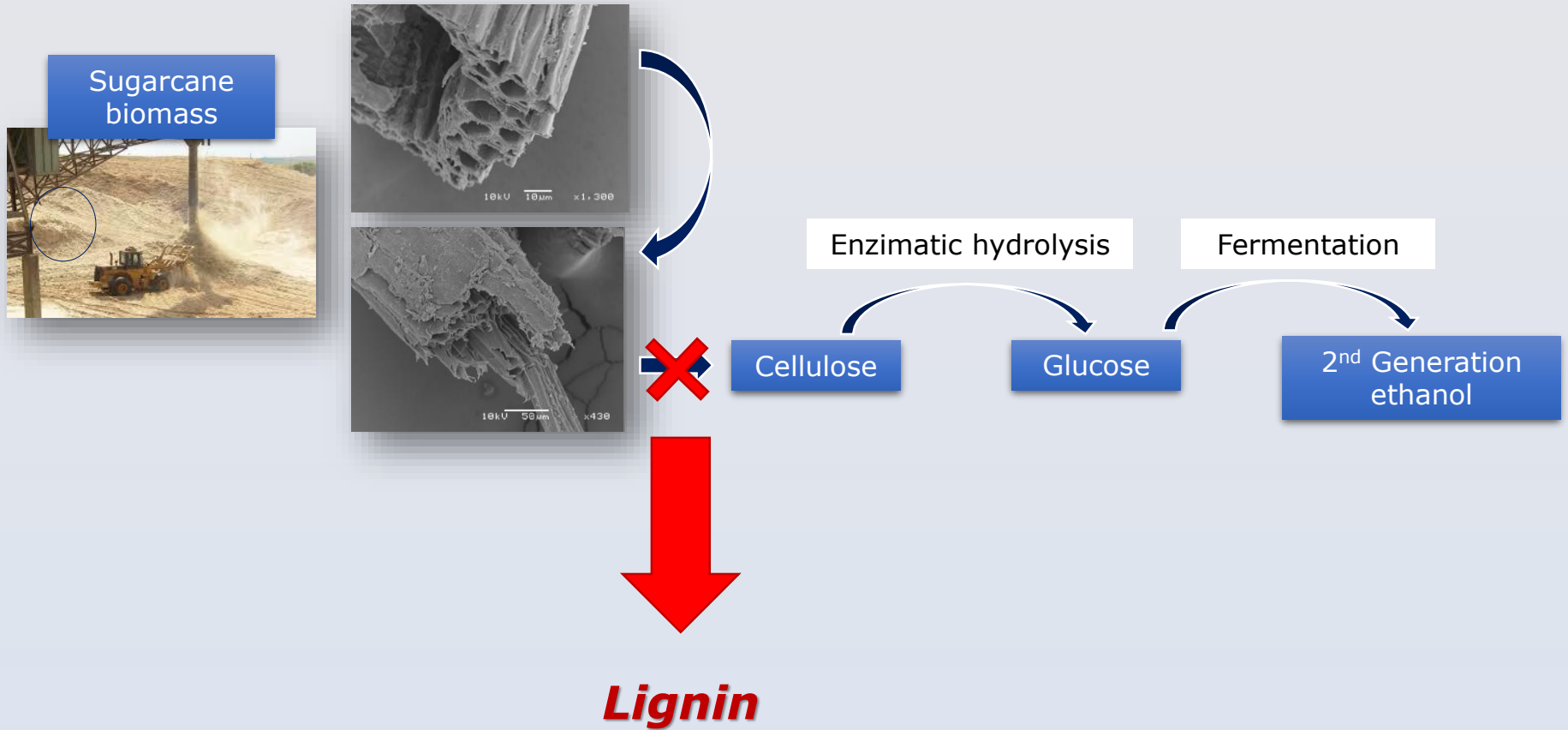
## **X-ray ptychography: plant sciences**

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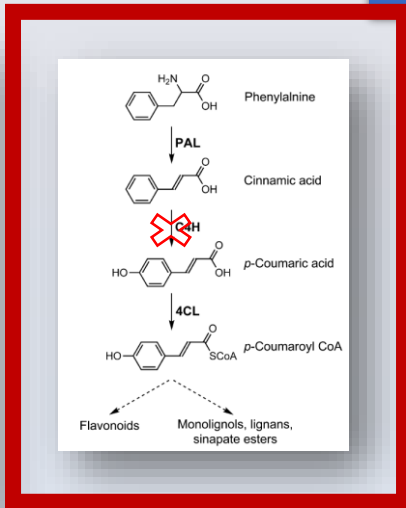




## Genetic manipulation (lignin subexpression)

Wild-type

Lignin mutant



Enzimatic hydrolysis

Fermentation

Cellulose

Glucose

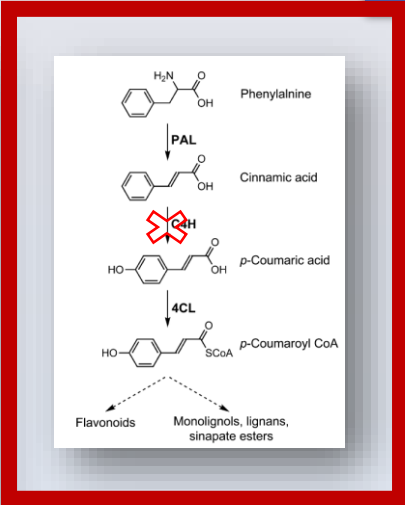
2<sup>nd</sup> Generation ethanol

- **Decrease in biomass recalcitrance**
- **Dwarfism**
- **Decrease in implosion resistance**



## Genetic manipulation (lignin subexpression)

Wild-type



Lignin mutant



- Altered cell morphology?
- Affected cellular types?
- Correlation between 3D data and implosion resistance?

Enzimatic hydrolysis

Fermentation

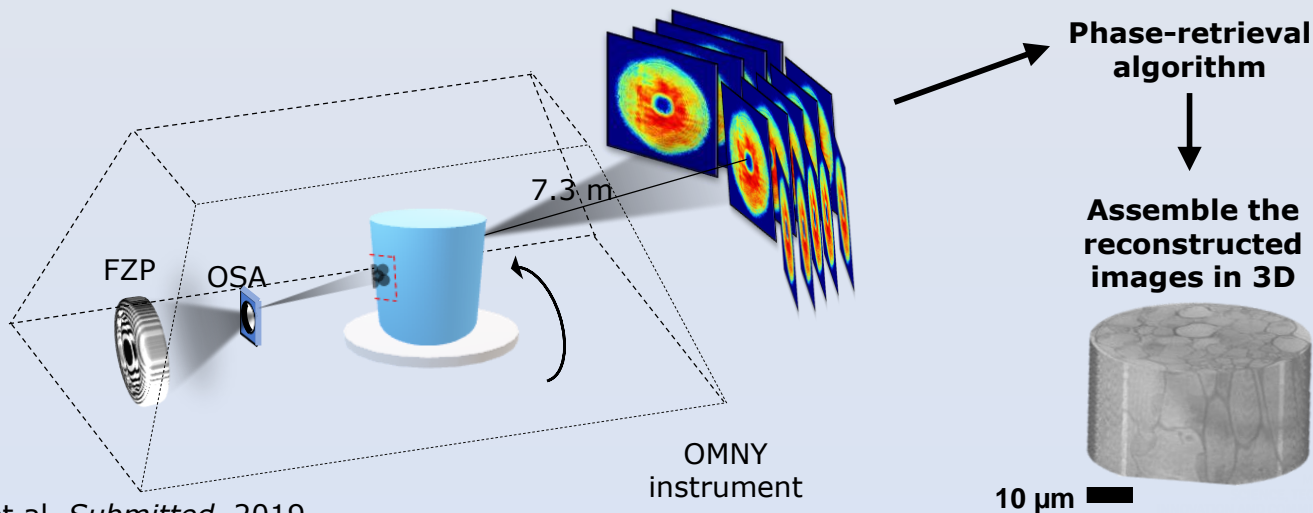
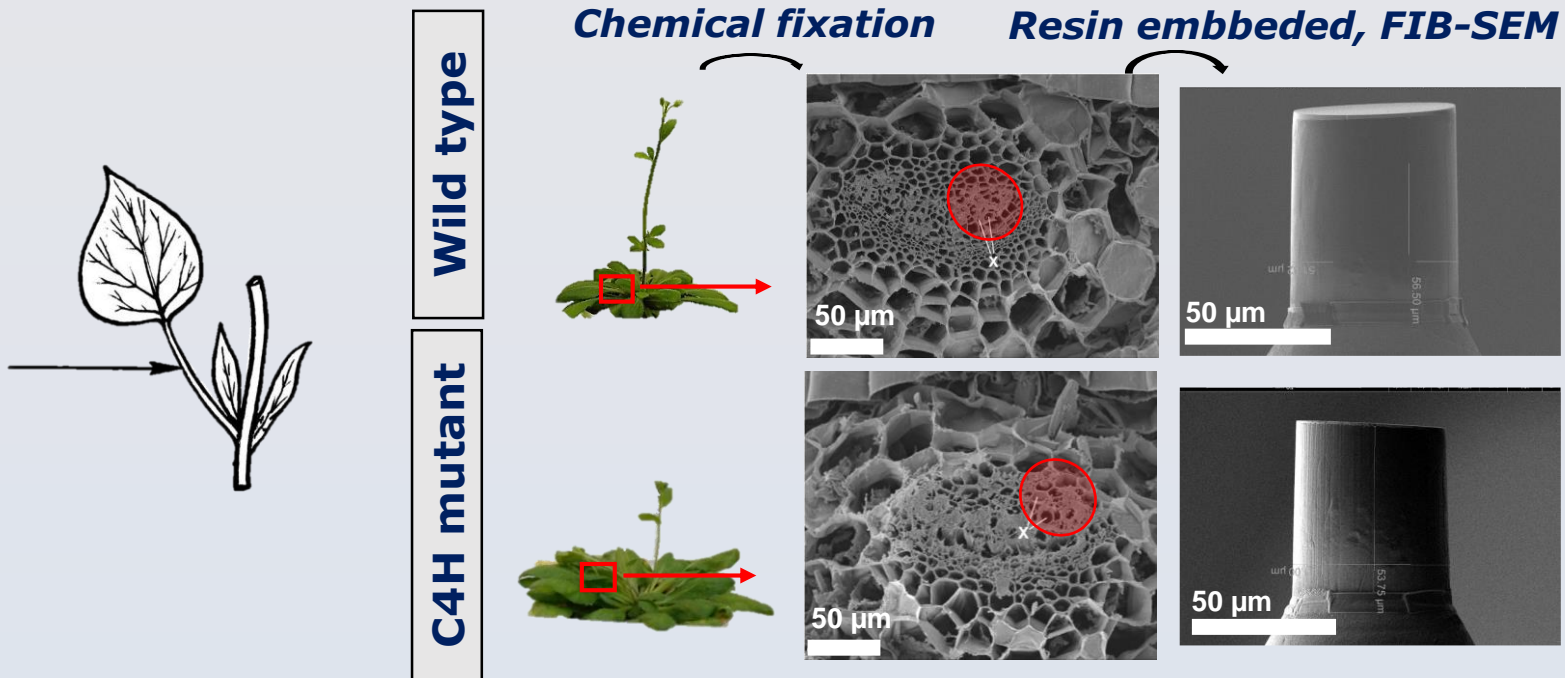
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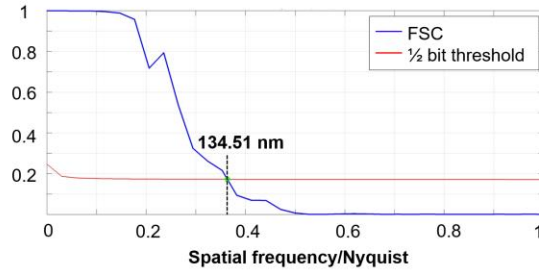
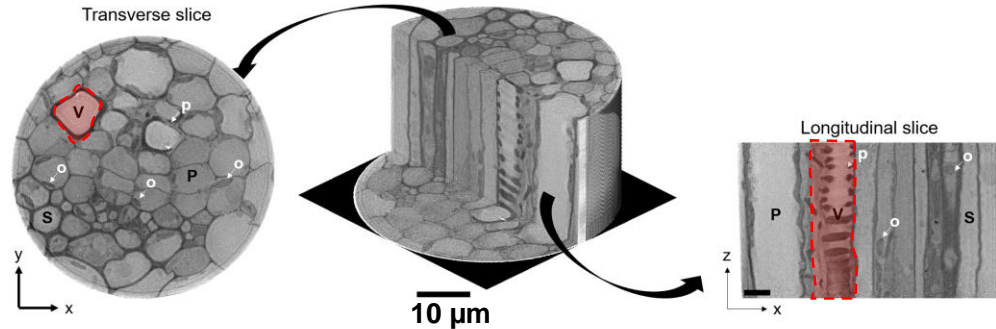
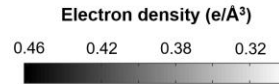
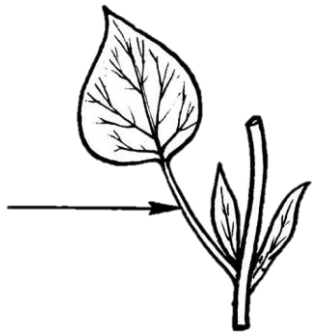
- Decrease in biomass recalcitrance
- Dwarfism
- Decrease in implosion resistance

# Ptychography X-ray Computed Tomography (PXCT) @cSAXS (PSI)

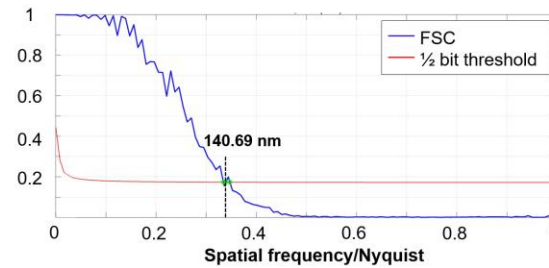
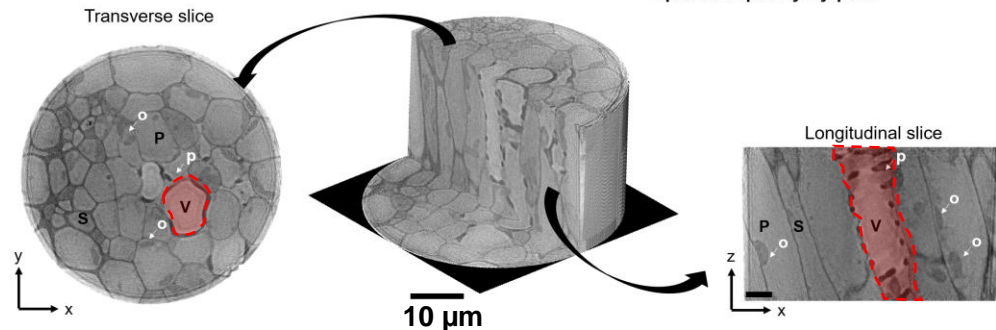
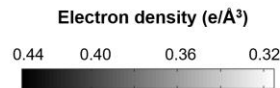


# Ptychography X-ray Computed Tomography (PXCT) @cSAXS (PSI)

**Wild-type**



**C4H mutant**



# Morphological dissimilarities and thickness distribution

*Vessel*

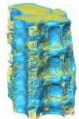
*Fibre (sclerenchyma)*

*Storage (parenchyma)*

**Wild-type**



**C4H mutant**



- Segmentation of different cellular types allows separation of hierarchical cellular compartments separation and accuracy morphology and thickness analysis

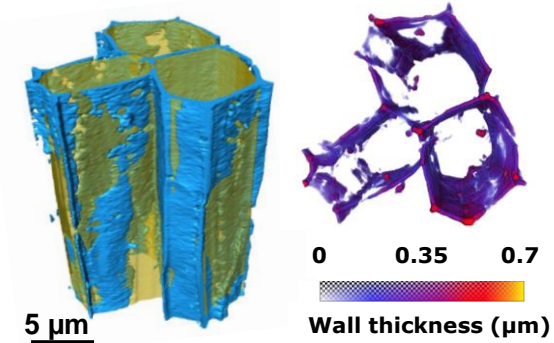
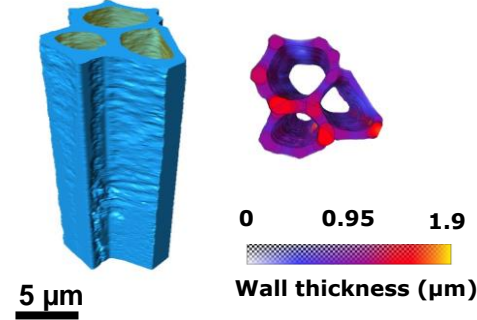
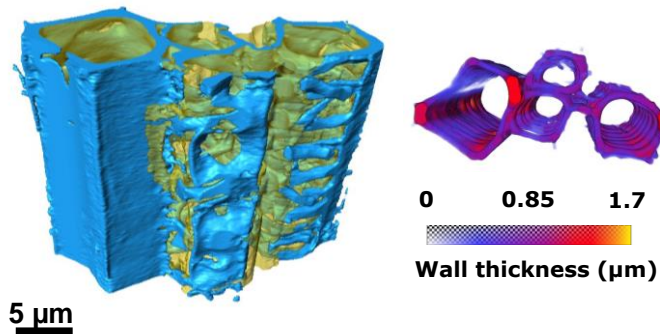
# Morphological dissimilarities and thickness distribution

Vessel

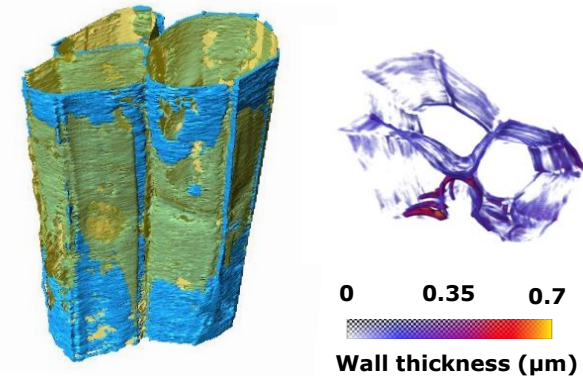
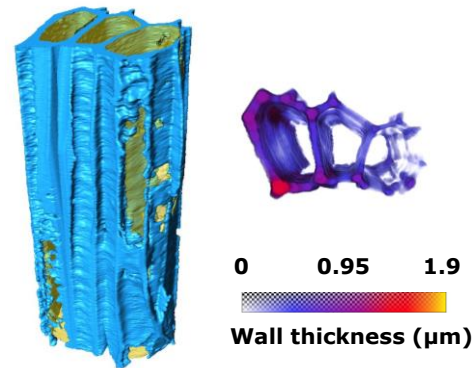
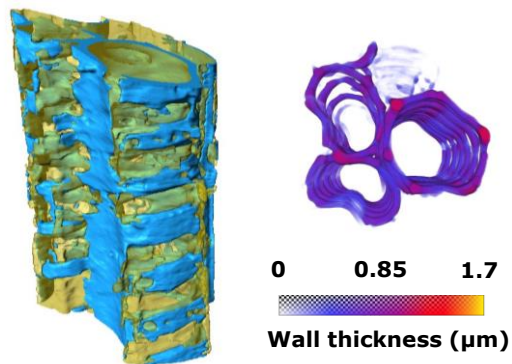
Fibre (sclerenchyma)

Storage (parenchyma)

Wild-type



C4H mutant

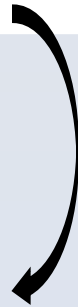
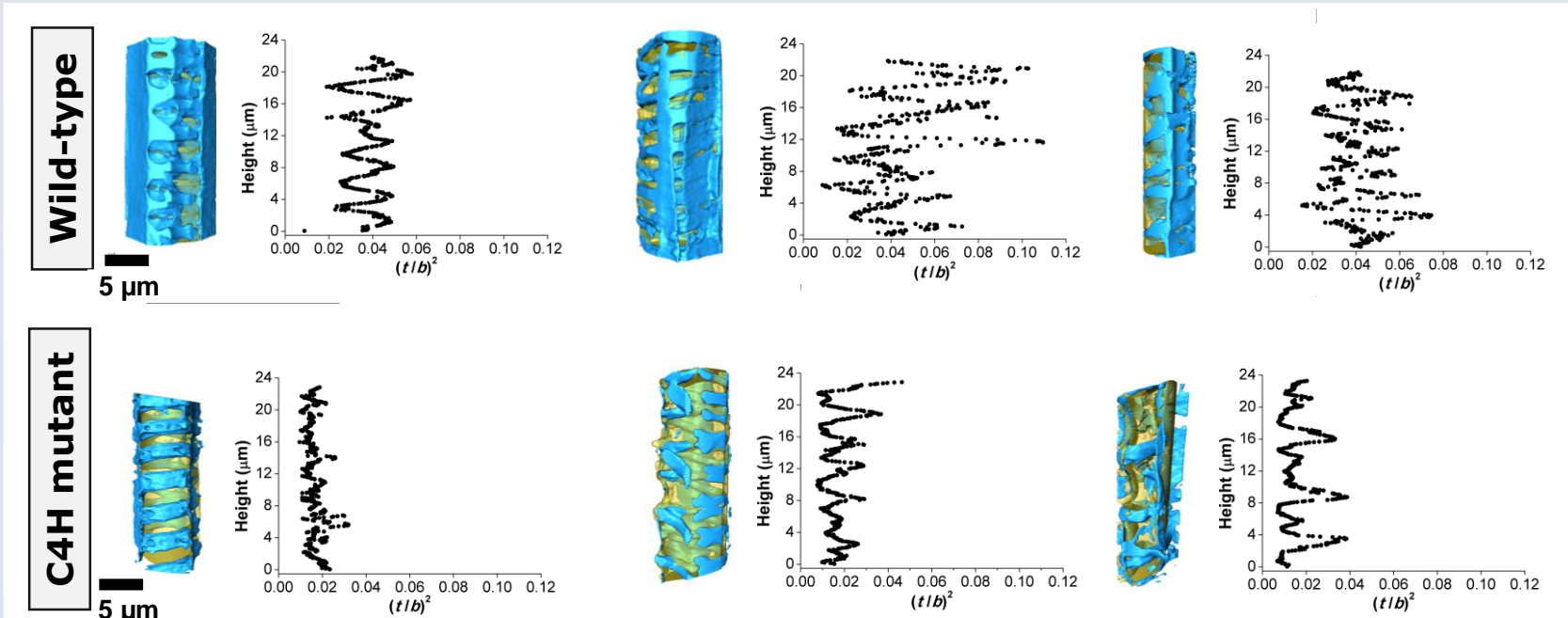
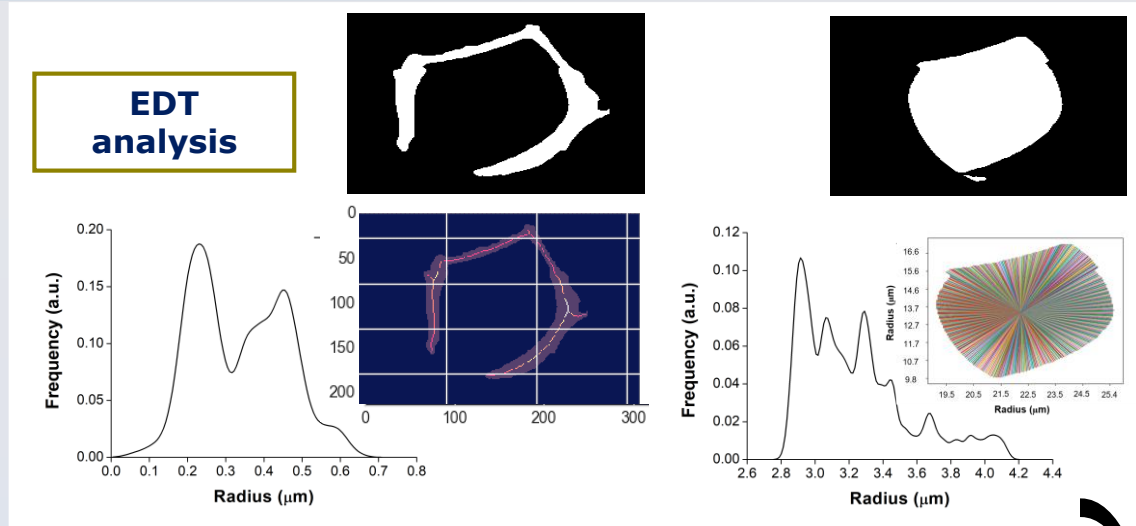


- Quantitative analysis of wall thickness distribution in the whole cell
- Support cells, such as sclerenchyma (fibres), are the most affected by the mutation causing decrease in  $\sim 60\%$  in the cell wall



# Implosion resistance evaluation in vessel (t/b)<sup>2</sup>

- Ratio between lumen and cell wall
- Complement 2D analysis: no average!
- Low amplitudes in (t/b)<sup>2</sup> of mutant might cause decreased stiffness in the cell wall



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## **Conclusion remarks**



## ***Coherent X-ray imaging to biological sciences***

- **Complementary to electron and x-ray microscopy/diffraction techniques**
- **Challenges: sample preparation, radiation damage and large volume of data processing**

## ***Ptychography***

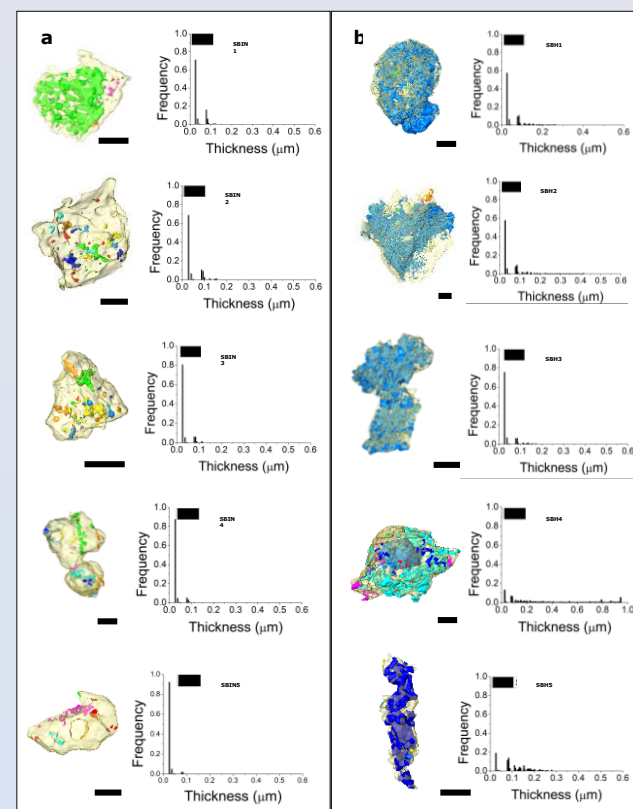
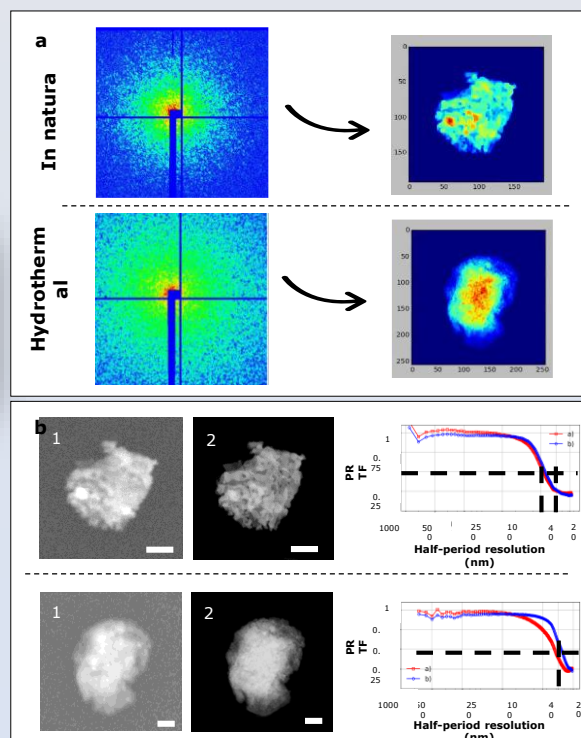
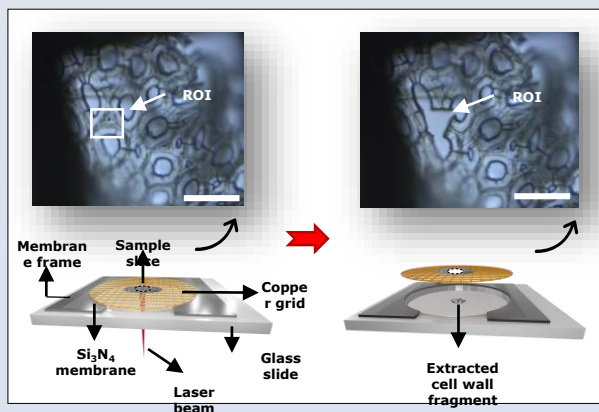
- **Allows to study extended biological tissues and extract information from different length scales**
- **Can bring quantitative analysis to plant sciences to improve the understanding of physiological processes**
- **New 4<sup>th</sup> generation machines: larger beam – larger sample; high-coherent flux- faster data acquisition.**

# Poster session

## More coherent x-ray imaging applied to biology

### Bagasse nano-structure modifications induced by hydrothermal pretreatment revealed by CXDI

Polo, C. C., Chushkin, Y.; Zontone, F.; Meneau, F.



## Poster

### **Coherent x-ray imaging @Sirius (Brazil)**

**Cateretê, the Coherent Scattering Beamline at Sirius, 4<sup>th</sup>  
Generation Brazilian Synchrotron Facility**

Meneau, F. et al.

### **Oral contribution**

**Operando Bragg CDI investigation of temperature hysteresis in  
CO oxidation on model gold catalysts**

Passos, F. et al.

## Cateretê group

Florian Meneau (Cateretê group leader)

Harry Westfahl Jr (scientific director)



Luciano Pereira



Denisele Flores  
Juliana Mayer



Paulo Mazzafera



Manuel Guizar-Sicairos  
Mirko Holler  
Ana Diaz  
Sarah Shahmoradian

