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X-ray Imaging of Water-conducting Pathways and Water Transport in Xylem Vessels of Vascular Plants

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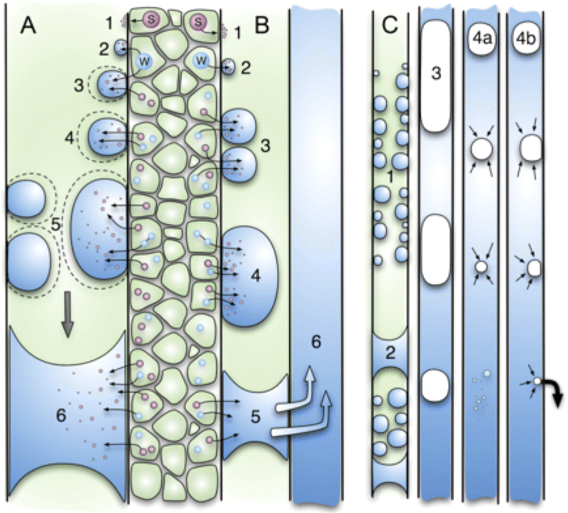
How do plants live so long?

- Annual modular growth
- Morphological structure is redundant and optimized
- Sectorized vascular system
- Root system replacement
- Protective structural system to defend from enemies
- Regeneration with stem cells (clone)

➢ The Great Basin bristlecone pine (*Pinus longaev*) of 4765 years old

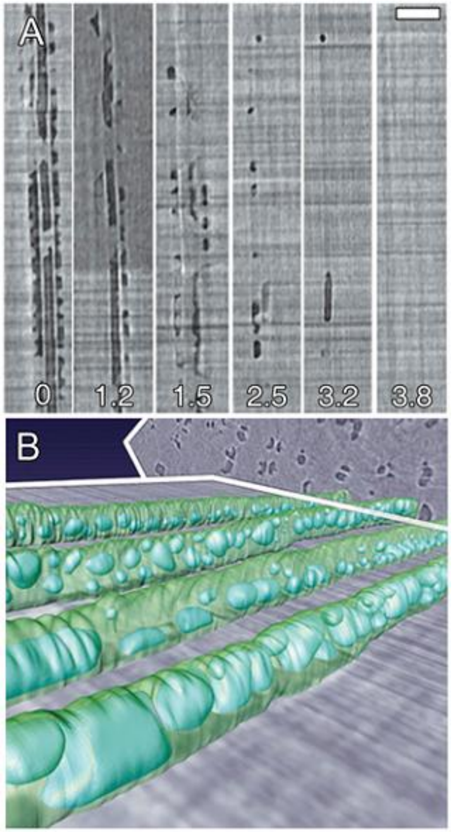
The coast redwood (*Sequoia sempervirens*): 116 m (Guinness World Records, 2006)



➢ Embolism repair model for grapevine (CR Brodersen et al. 2010)

Introduction

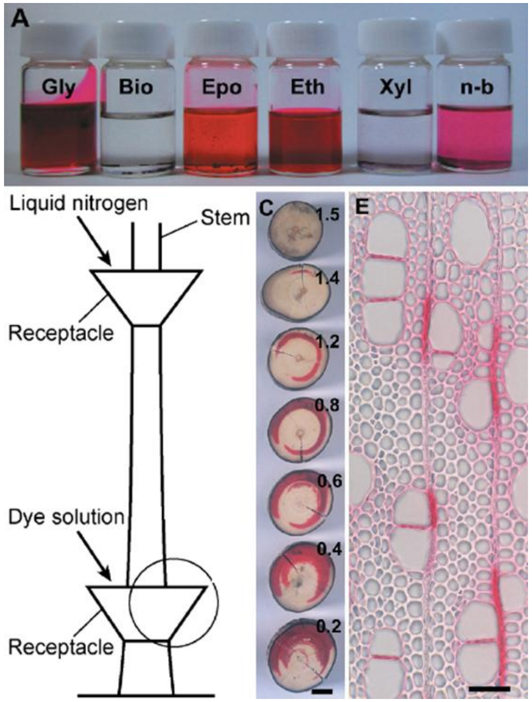
- ➢ How to *in vivo* visualize water transport inside an opaque vascular plant?



Brodersen et al, *The dynamics of embolism repair in .., Plant Physiology* (2010) **(MRI)**

- ➢ Conventional approaches are difficult to observe **dynamics** of sap flow in xylem vessels (**plant hydraulics**)

- ➢ X-ray imaging tech. has micro-scale spatial resolution at temporal resolution of tens ms.

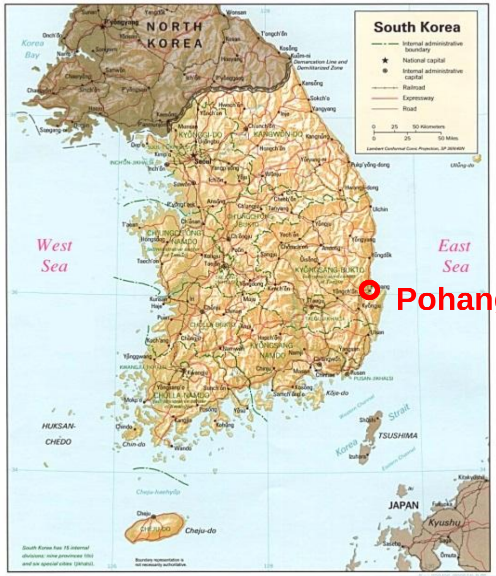


Sano et al, *Visualizing water-conduction pathways of living trees, Tree Physiology (2005)* **(Dye + Cryo-sectioning)**

- ➢ X-ray micro-imaging technique was developed to investigate the morphological structures and hydrodynamic phenomena in xylem vessels of vascular plants

Comparison of Laser and X-ray light source

	Laser	X-ray
Wavelength	300 ~ 1000nm	0.01 ~ 3 Å
Resolution	> Micro scale	~ Nano scale
Imaging	Mie-scattering	Phase-contrast
Transmittance	No	Yes



Pohang Light Source (PLS)

- High energy / 3.0 GeV
- White beam line (infrared ~ hard X-ray)
- Intensity : ~10^{17}photons/sec/mrad^2/mm^2
- Pulse width 10nsec / interval~1μsec
- Polarization : linearly polarized

X-ray free electron laser (4^{th} generation)

- Wavelength: 10^{-9} ~ 10^{-12} nm
- 10GeV electron accelerator: 10^6 times brighter than 3^{rd} generation
- Temporal resolution : 10^{-15}sec.

X-ray micro-imaging of sap transport in monocot plants

- In vivo visualization of morphological anatomy of xylem vessels
- Water-rise kinetics : trace the water-air meniscus in xylem vessels during water-refilling process after dehydration.

Experimental system

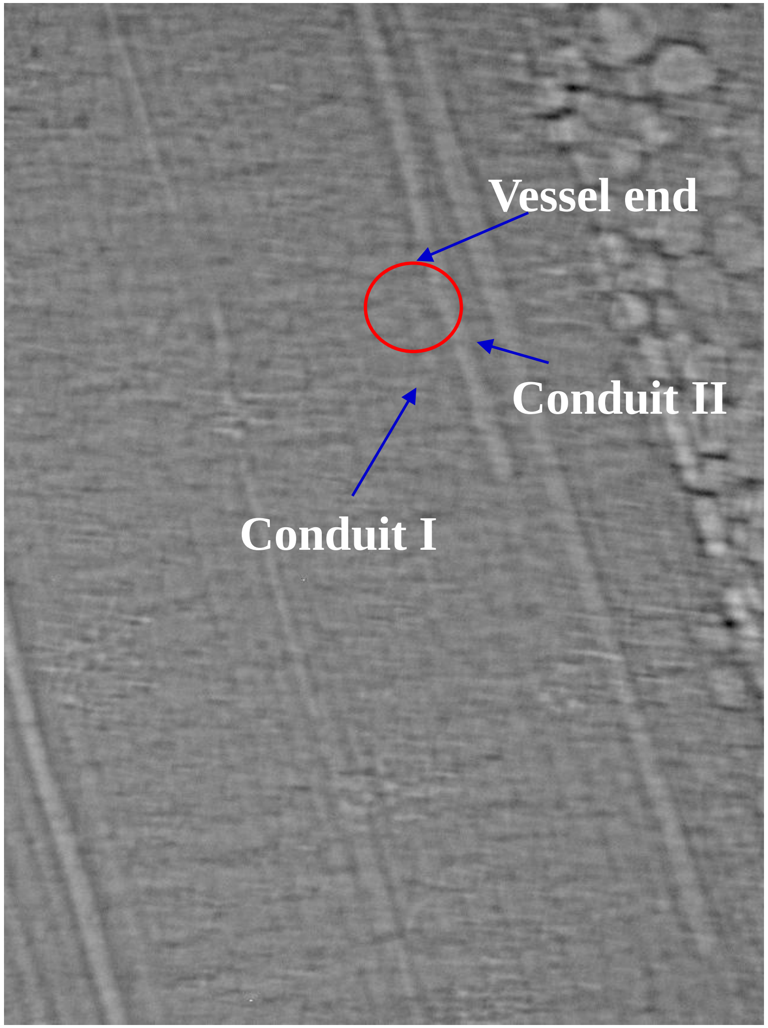
- X-ray : White beam
- CCD camera : Cooled CCD
- Objective lens : 10× zoom lens
- Field of view : = 858 μm × 686 μm
- Mechanical shutter (exposure= 4ms)
- Test specimen: Xylems of bamboo tree

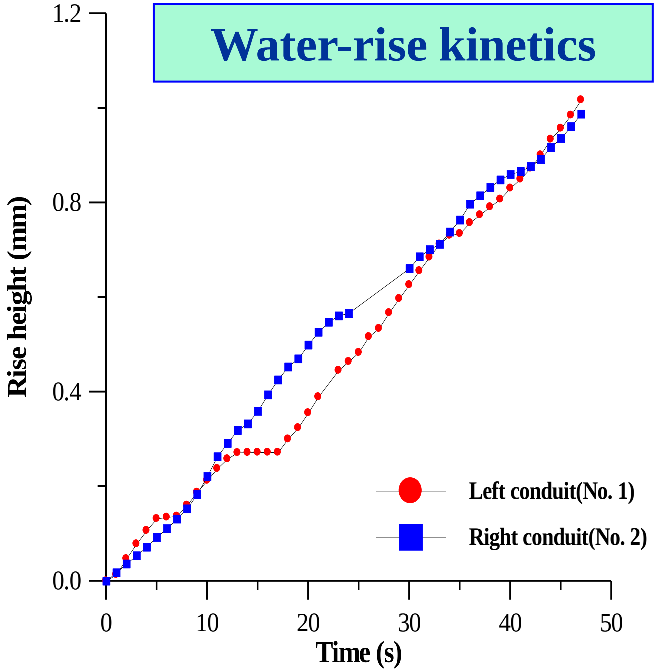
Preparation of test specimen

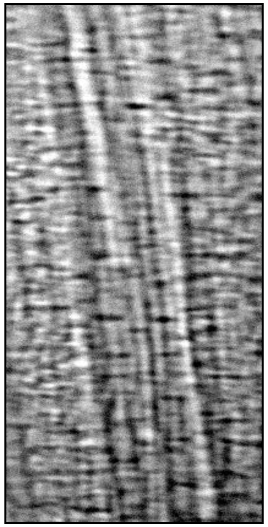
- A bamboo branch was cut from a garden
- put in a vase for one day before experiment
- A leaf was excised for experiment

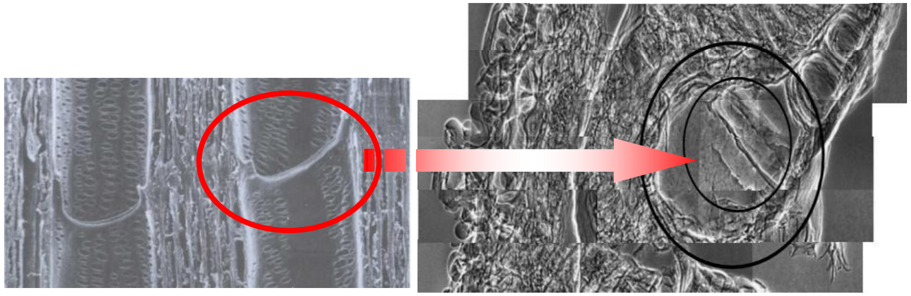
Water-rise kinetics

X-ray imaging of sap flow in xylem vessels of a bamboo leaf







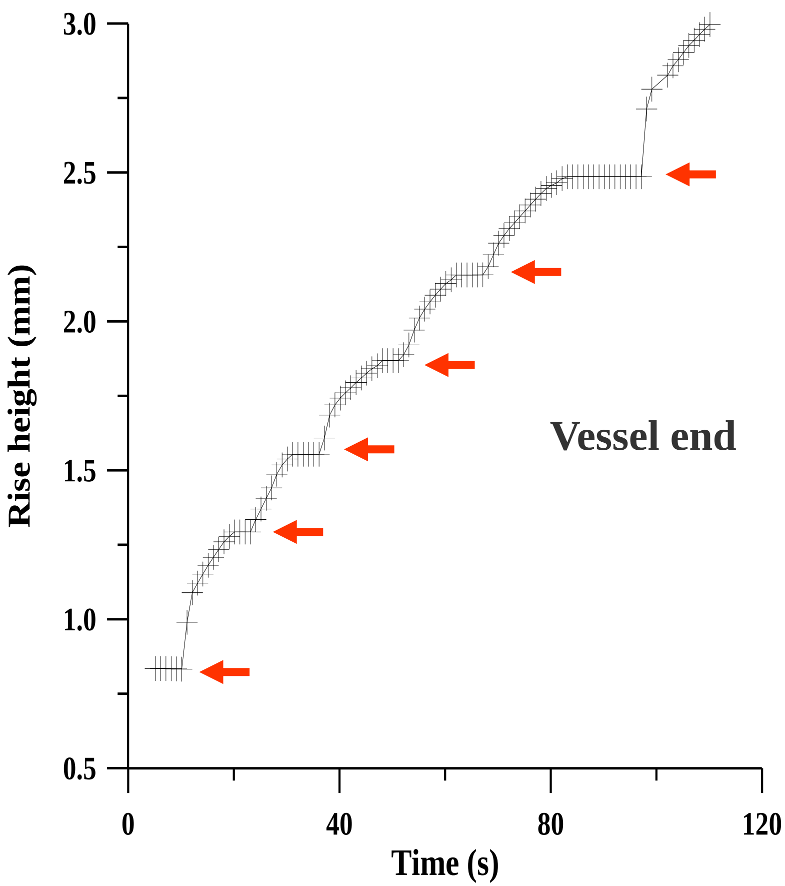


[Perforation plate] [Vessel end]

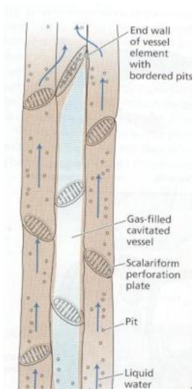
SJ Lee & YM Kim, Annals of Botany, Vol.101, pp.595-602, 2008

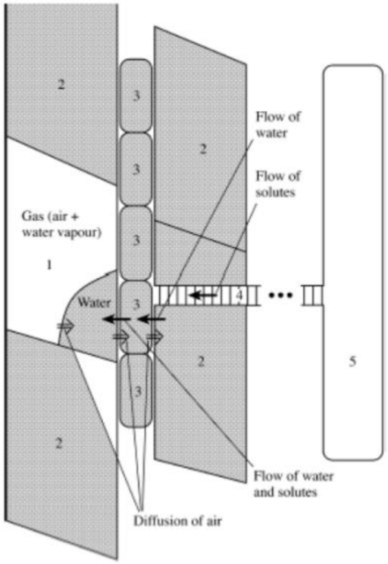
Water-rise kinetics in a xylem vessel of bamboo stem

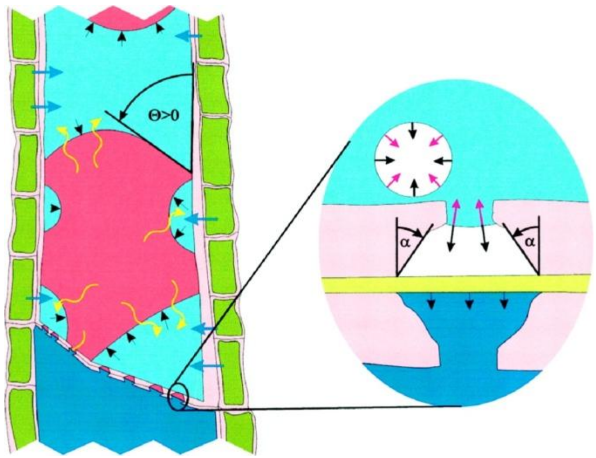
Water-rise kinetics & bubble removal suggest that the vessel end works as a hydraulic valve ($P_{accmulation} > P_{threshold}$)



[Schematic of refilling vessel]
[M. Perämäki, Ph.D Thesis, 2005]







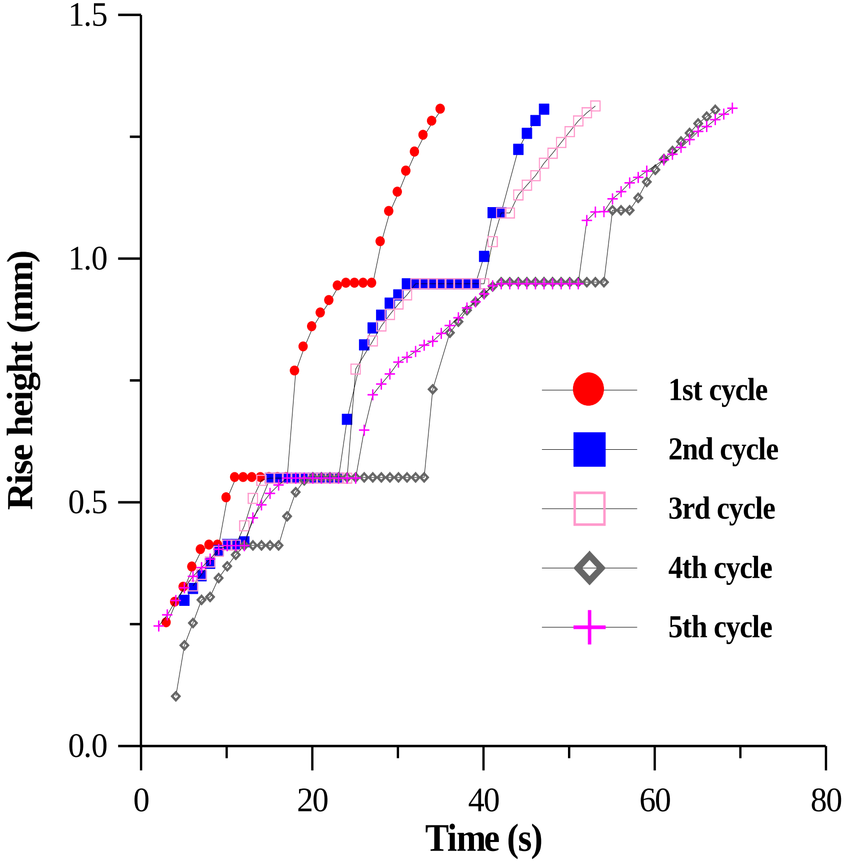
(a) Embolized vessel (b) Bordered pit geometry

Hydraulic compartmentalization model for embolism repair (Holbrook & Zwieniecki, 1999)

Validation of embolism repair models!!

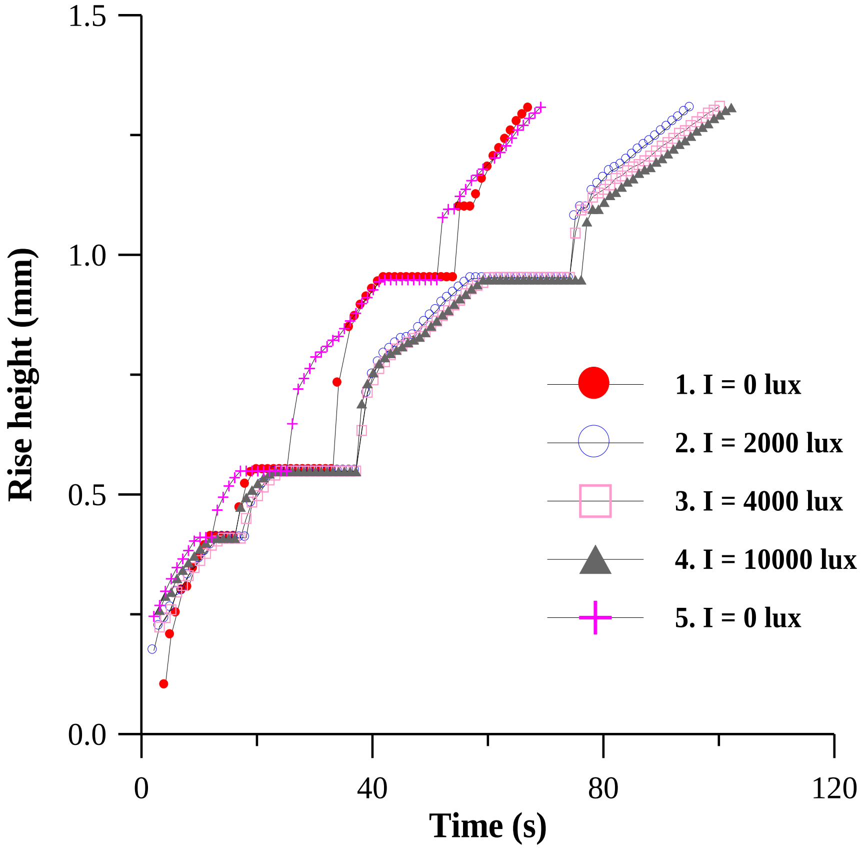
Effects of repeated water-refilling cycle & light intensity on water-rise kinetics

Effect of water-refilling cycles



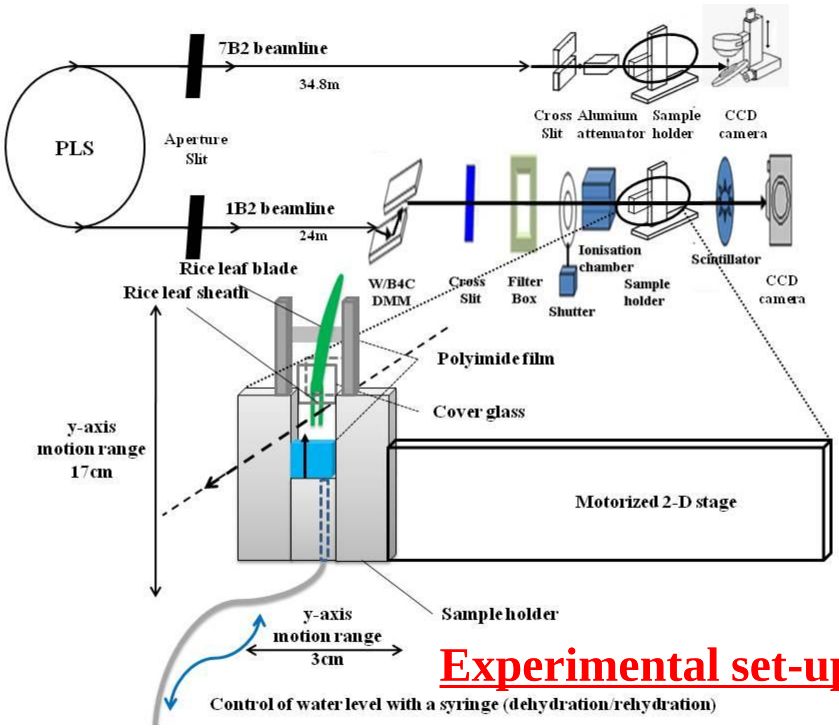
Repeated cavitation weakens the ability to refill water in xylem vessels

Effect of light intensity

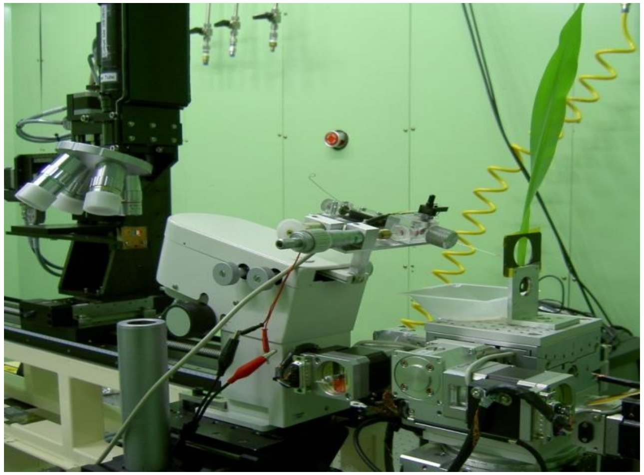


Darkness facilitates water refilling in embolized xylem vessels

Water-rise kinetics in xylem vessels of rice leaf

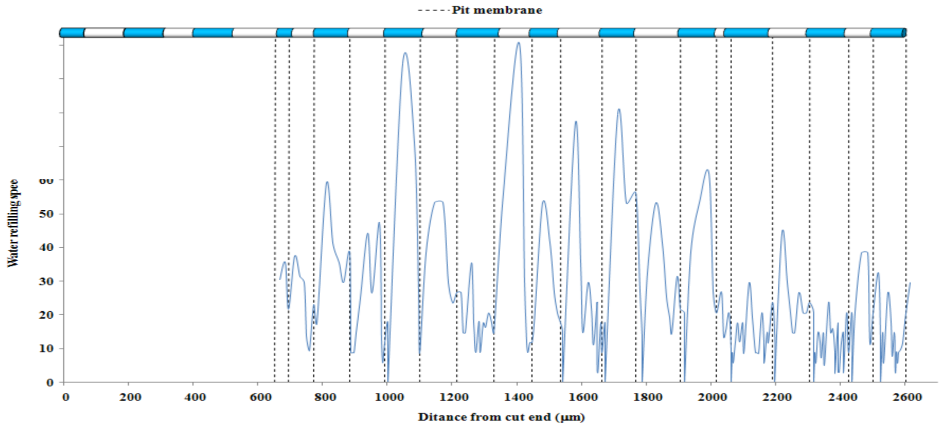


Experimental set-up



Synchrotron X-ray imaging technique

- High temporal & spatial resolution (µm)
- Ideal to visualize xylem of monocot plants (simple parallel vascular organization)
- Real-time imaging of water-refilling process



Speed of water-uptake (10µm/s ~ 3mm/s)

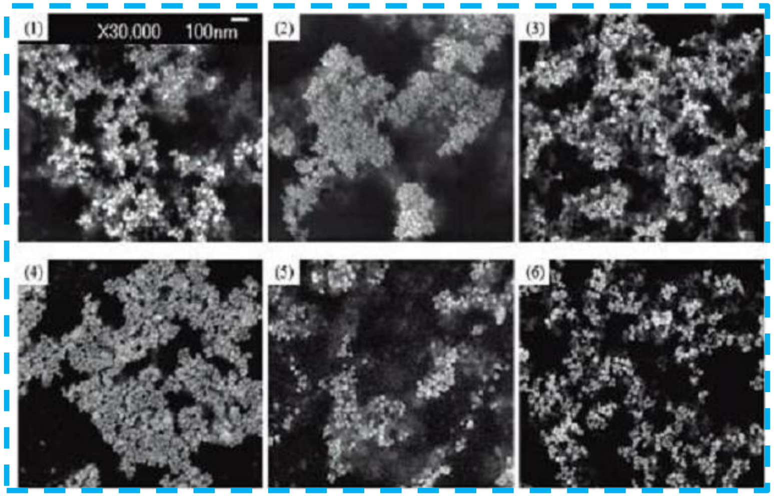
Use of AuNPs as tracer particles of sap flow in vascular plants

➢ Limits of X-ray micro-imaging method :

- **Sample excision is required to track air-water meniscus**
- **Distinctive flow tracers are seeded to apply a PIV or PTV technique**

➢ Why gold nano-particles (AuNPs) ?

- **Superior X-ray absorption rate, compared to surrounding tissues (x100) and even contrast agents used in clinical diagnosis (iodine, x2.7)**
- **Typically bio-compatible material**
- **Various sizes, shapes, and surface properties available.**
- **AuNPs used : hydrophilic, $\phi = 20$nm**



Ahn et al, *Gold nanoparticle flow sensors designed for dynamic X-ray imaging in biofluids, ACS Nano* (2010)

Use of surface-modified AuNPs as flow tracers in sap flows

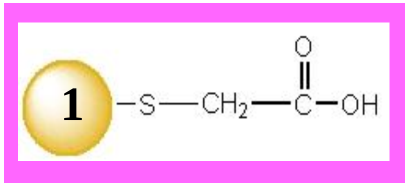
Chitosan (Mw:3,000~85,000 Da)

Chitosan	Molecular weight (Da)	Viscosity (Pa.s)	Deacetylation (%)
#1	3,000	<0.1	85.47
#2	5,000	<0.1	85.50
#3	20,000	4×10^7	95.56
#4	30,000	2×10^8	85.51
#5	85,000	5×10^8	85.36

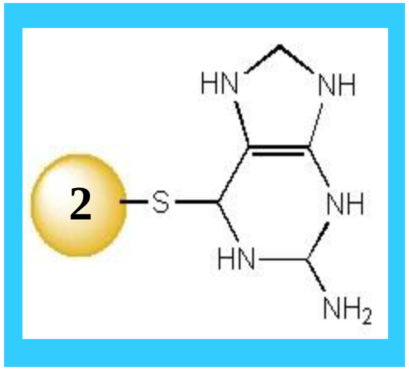
Au ions concentration

[I] 6.3×10^{-3} mmol Au
[II] 3.2×10^{-3} mmol Au
[III] 6.3×10^{-2} mmol Au

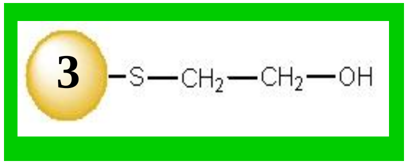
Surface-modified AuNPs



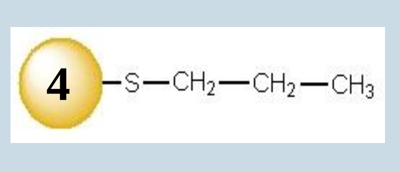
Acid



Base



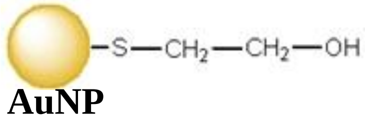
Hydrophilic



Hydrophobic

S Ahn, SJ Lee et al., ACS Nano, 4, 3753-3762, 2010
S Ahn, SJ Lee et al., Acta Biomateria, 7, 2139–2147, 2011

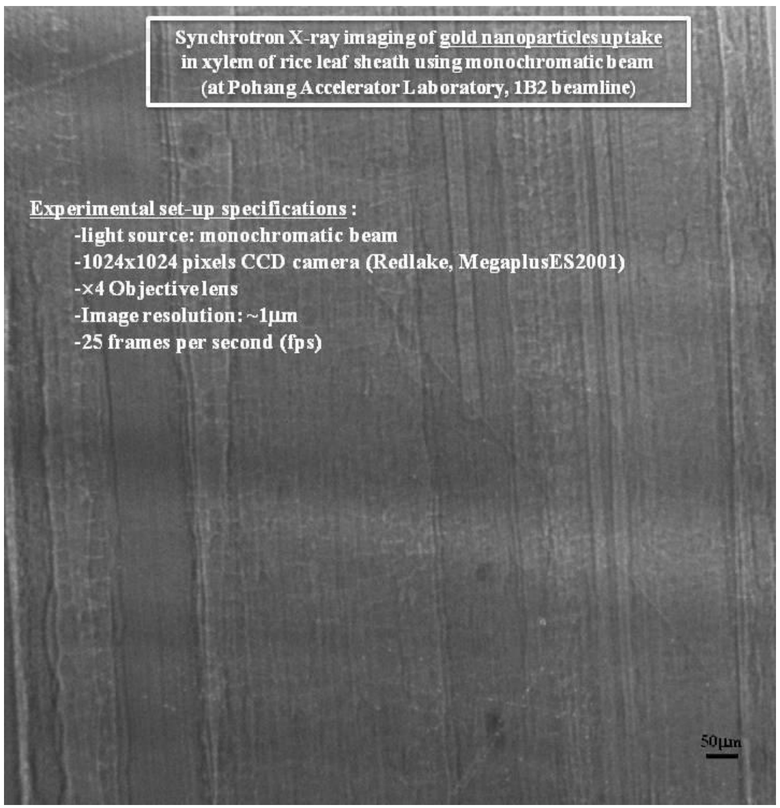
Diagnosis of bio-dynamics of sap flows using AuNPs

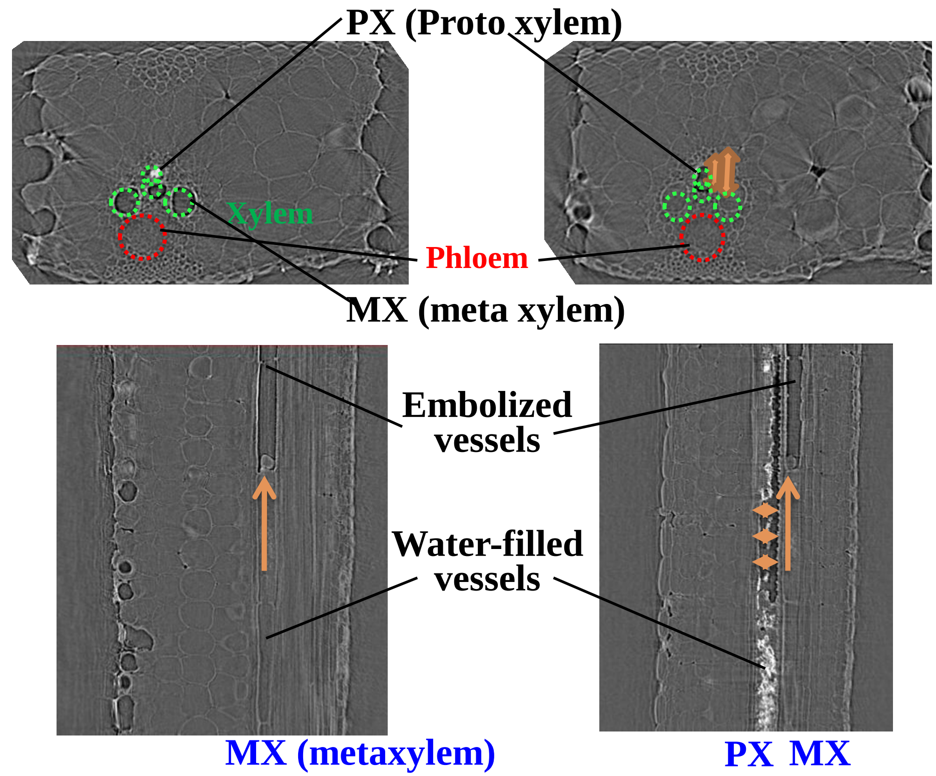


Hydrophilic AuNP 2-mercaptoethanol ($SH\text{-}CH_2CH_2OH$)

SS Ahn, SJ Lee et al., ACS Nano, 4, 3753-3762, 2010

3D vascular bundle organization in monocot leaves





Refilling process

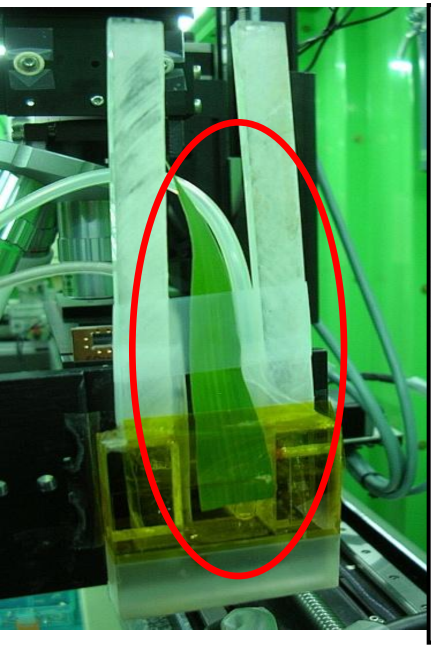
PX: Lateral (radial) movement

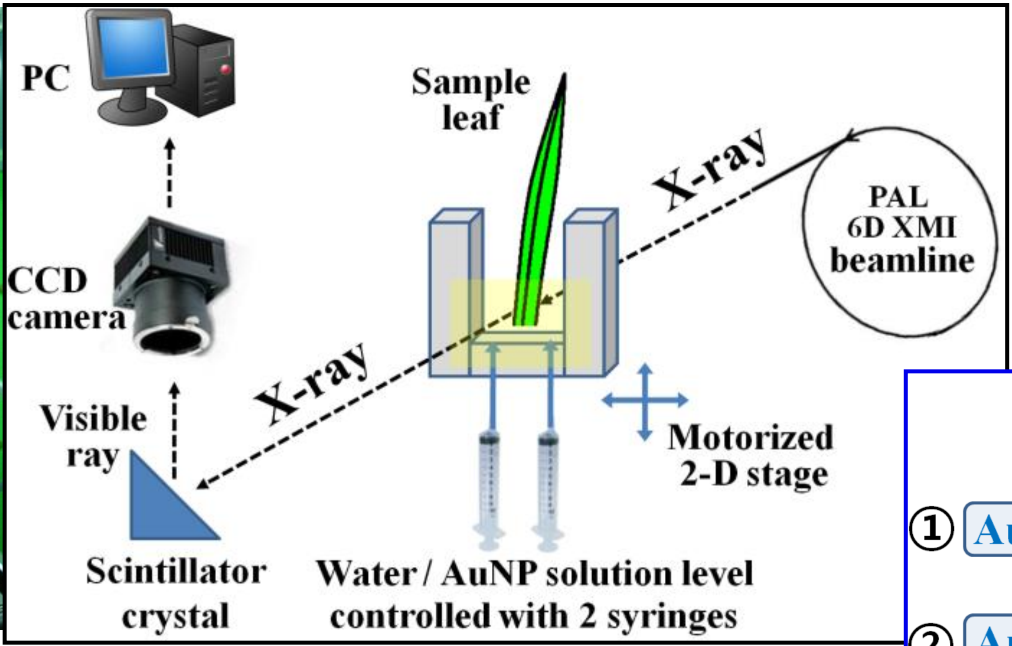
Hydrophilic AuNPs to visualize sap flows in xylem vessels of rice leaf

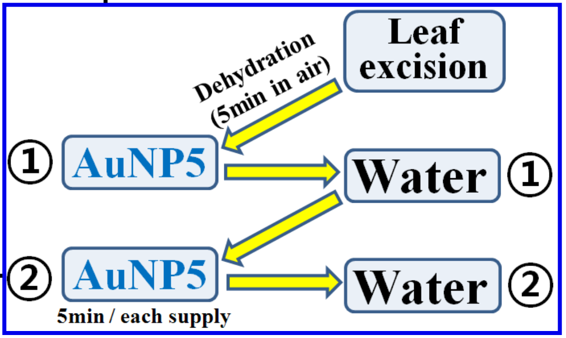
HK Kim & SJ Lee, New Phytologist, 188, 1085-1098, 2010

Feasibility of AuNps as flow tracers of sap flows in plants

- 3 excised monocot (maize /rice / bamboo) were tested

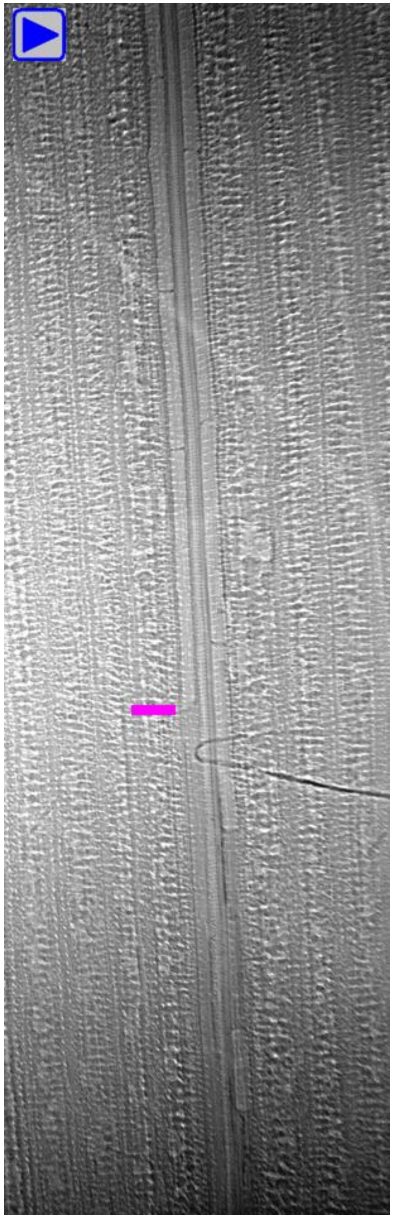


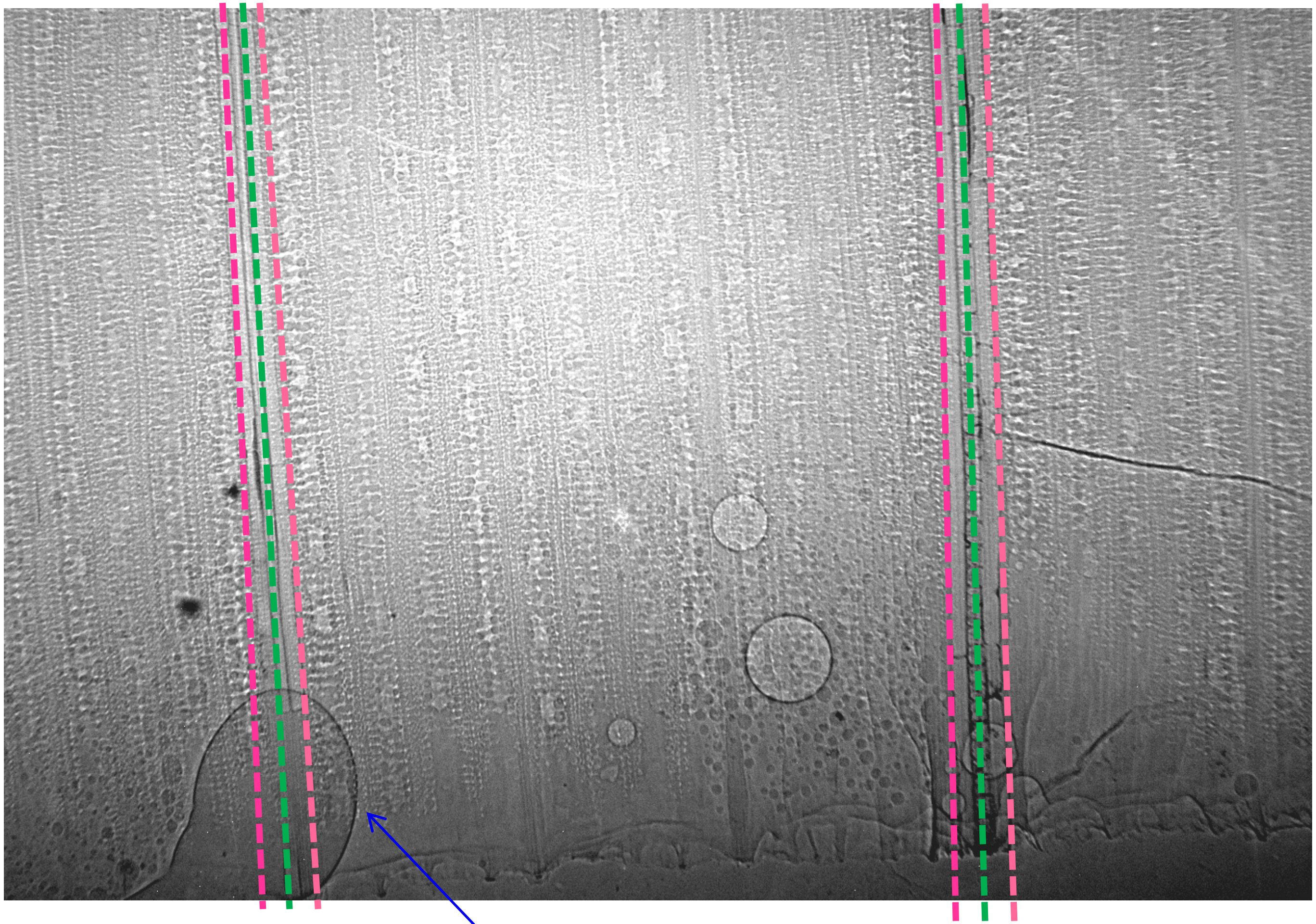




- 6D X-ray microscope beam line of Pohang Light source (3GeV)
- Coherent X-ray beam is converted via scintilater crystal, and magnified by a microscope lens.
- Alternative supply of AuNP solution and distilled water ⇒ repeated observation at the same xylem vessel. ⇒ minimize the variances in individual sample characteristics.

Dynamic imaging of sap transport in a maize leaf



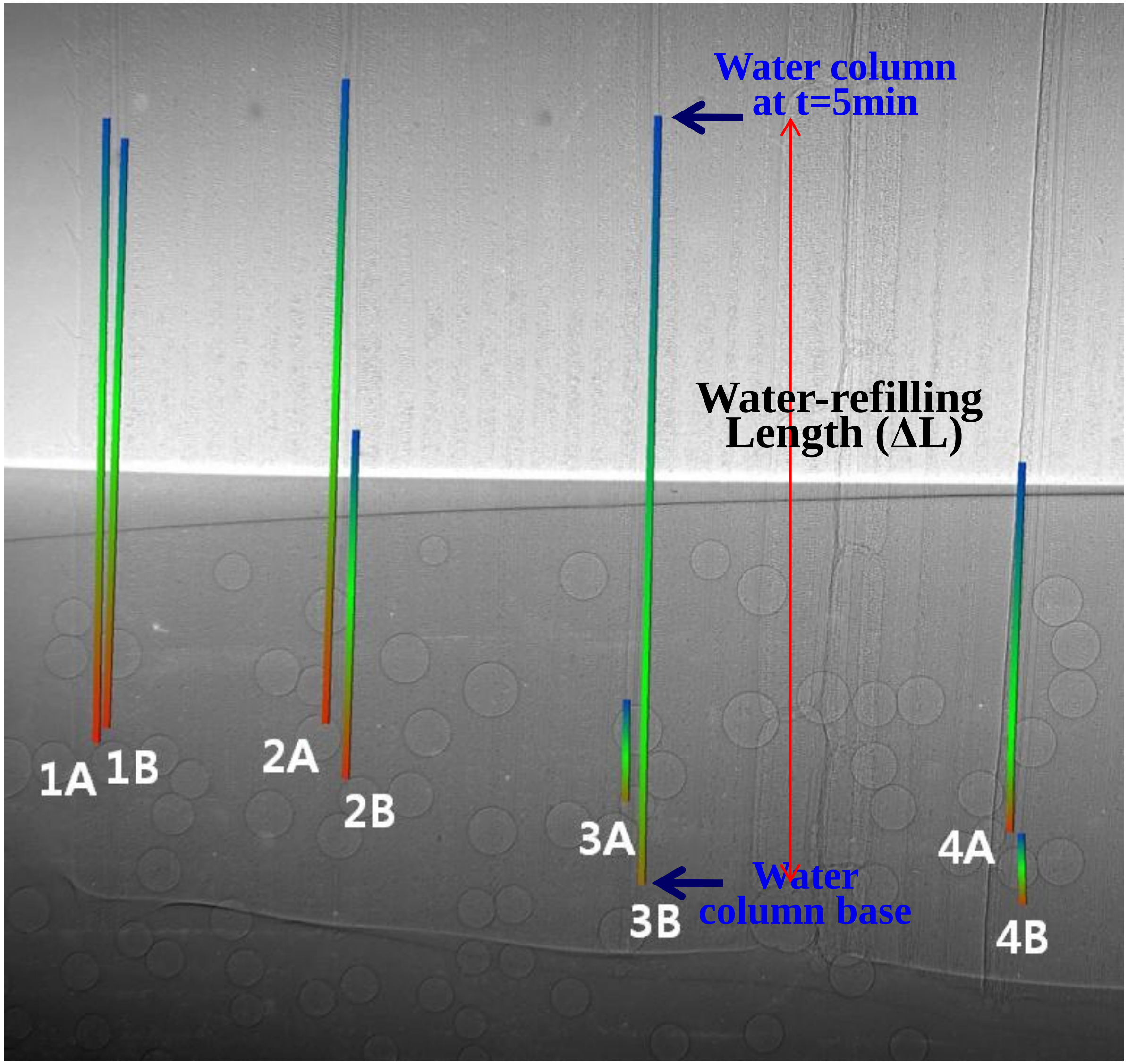


- Images were captured at 5 fps with 10x (<1μm/pixel)

*Meta & Proto xylems in a vascular bundle

Water droplets on the leaf surface

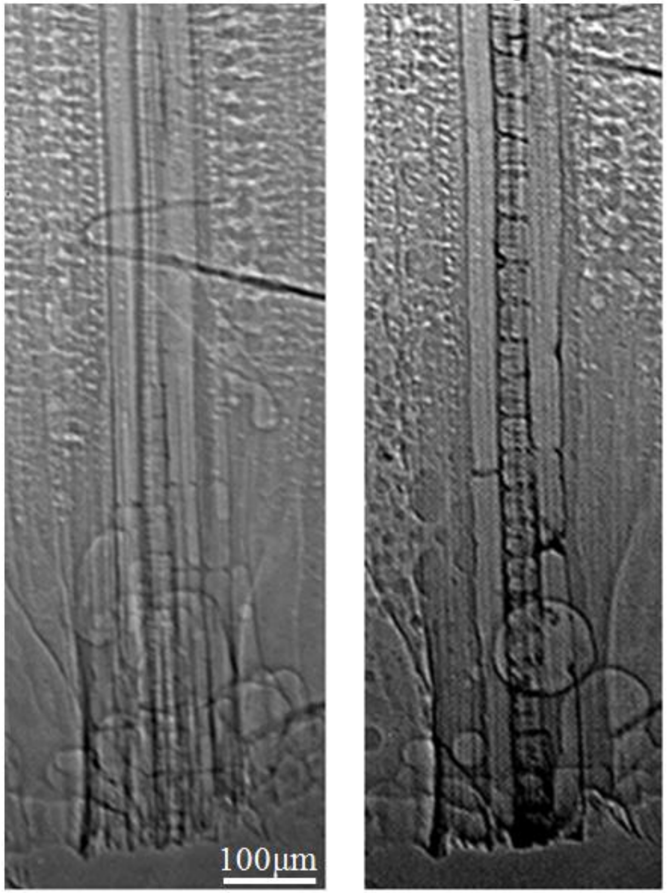
Measurement of water-refilling speed in a rice leaf



- **Bamboo samples were recorded at 5 fps, 5x (7.0x4.6mm FOV)**
- **Less missing data and multiple data acquisition**
- **Large variations of water-rise speeds in the same sample .**
- **Evaluate water-refilling speed : Σ ΔL(<u>refilled length per time elapsed.</u>**

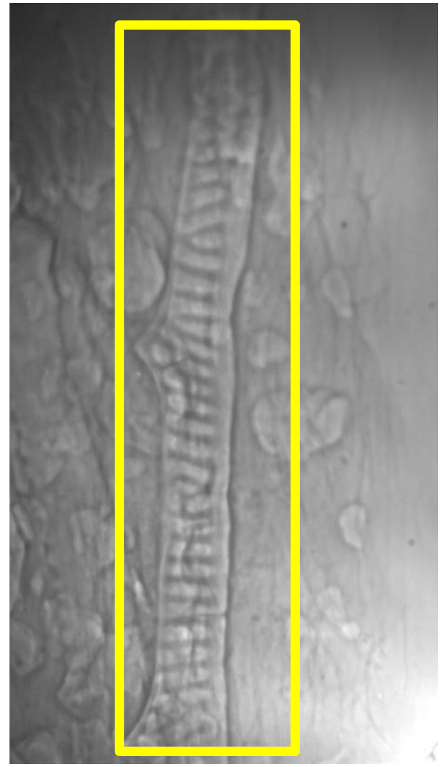
Xylem wall surfaces are stained by AuNPs

X-ray micro image



Before ⇨ **After**
1st AuNP supply

X-ray nano image



SEM image

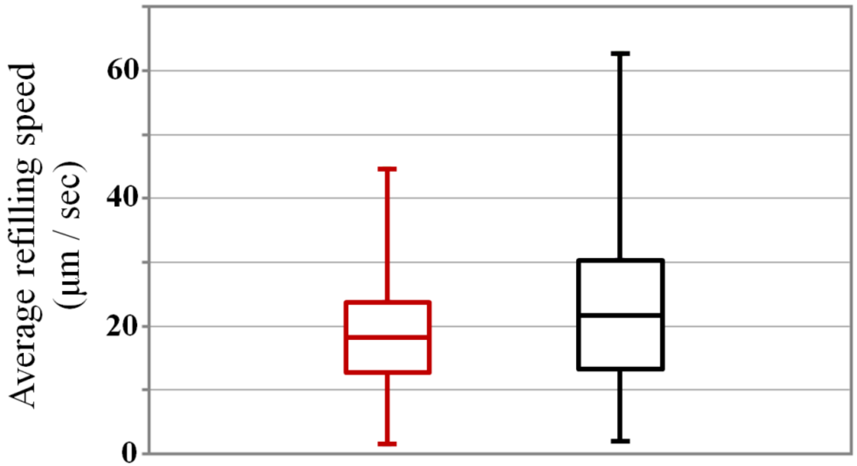
X-ray nano image of AuNPs-stained protoxylem vessel.
Morphological structure is similar to the SEM image

- **Anatomical features of xylem vessels and their internal morphological structures are distinguished.**

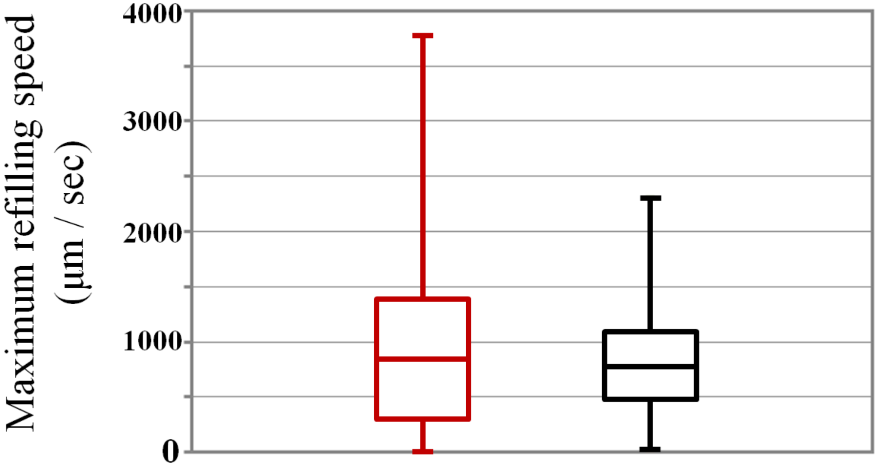
- **AuNPs are uptaken into xylem vessels during the rehydration of AuNP solution and they stain the wall surfaces of xylem vessels.**

Average & max. water-refilling speed in maize leaves (n = 47)

< Average refilling speed >

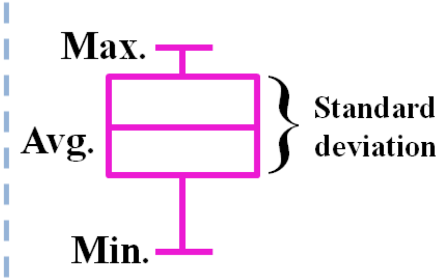


< Maximum refilling speed >



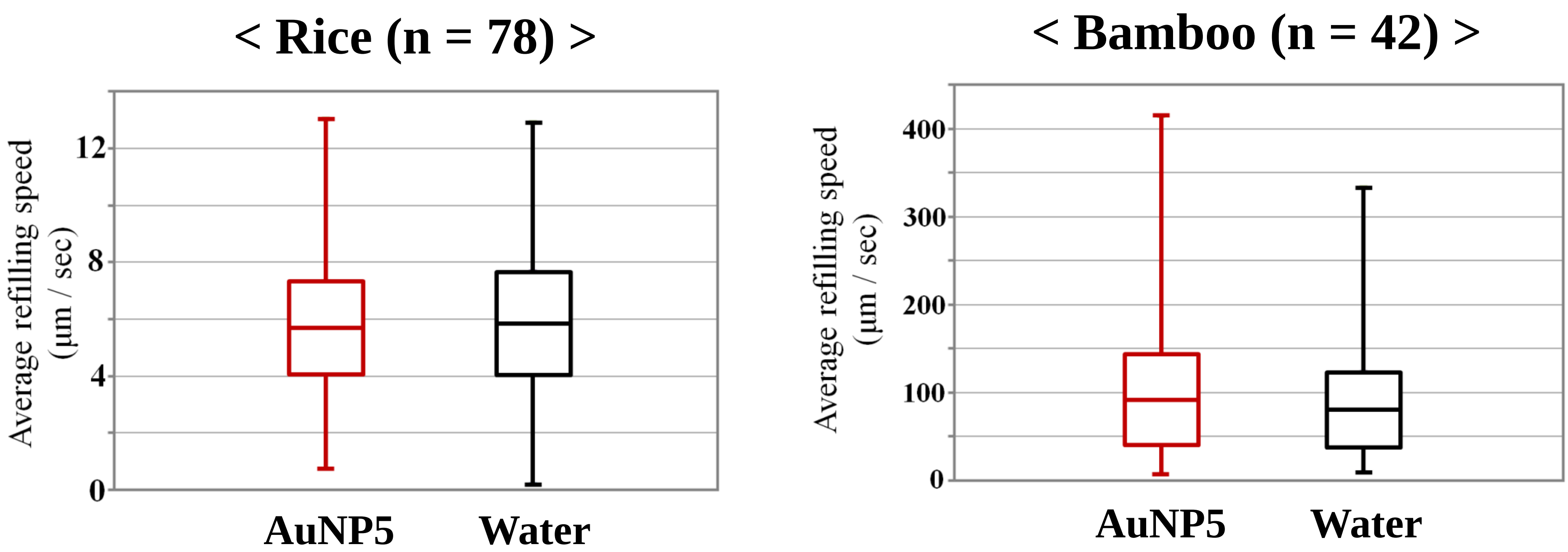
- The average water-refilling speeds of AuNP solution and distilled water are similar (measured by air-water meniscus tracking).

- Variations in the min. and max. speeds are wide. However, this is a typical feature observed in the water-refilling experiment using excised plant samples. Sap hydraulic characteristics of individual conduits vary widely.



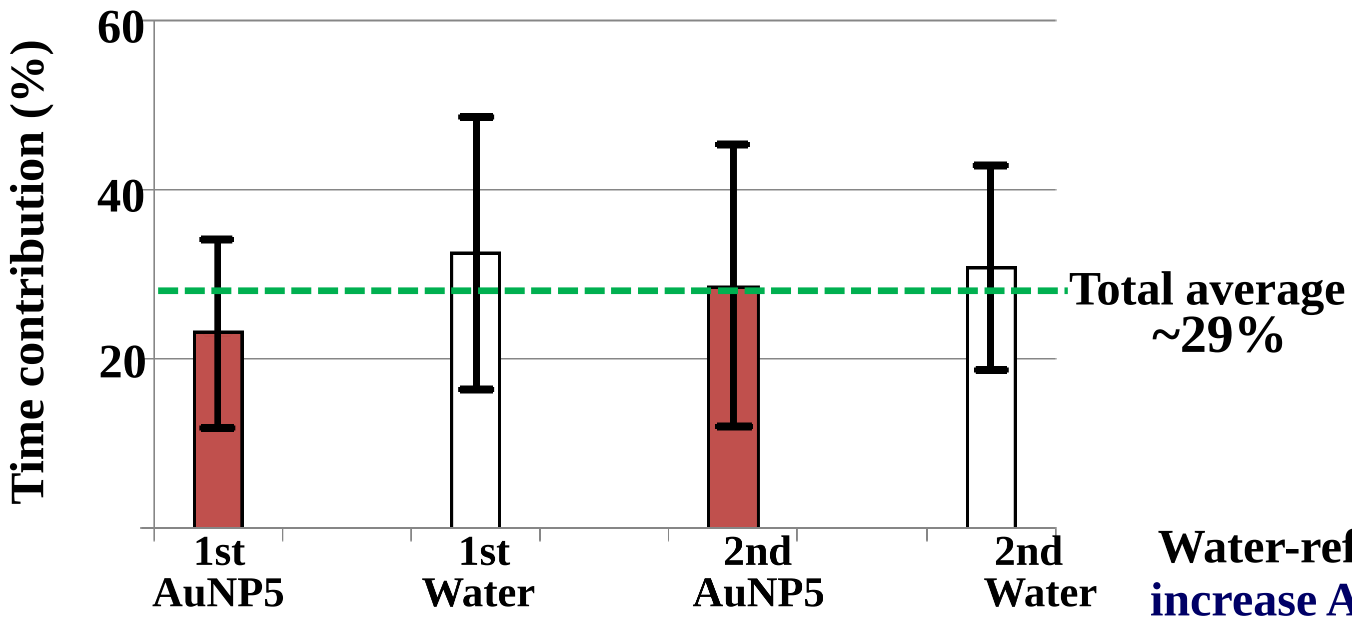
< Bar plot >

Comparison of average water-refilling speeds in plant leaves



- **The average water-refilling speeds of AuNP solution and distilled water are nearly identical.**
- **The obstructive effect of AuNPs supply to the water-refilling speed of xylem-vessels is not so significant during the X-ray imaging experiment.**

Effect of water-stoppages at perforation plates to water refilling

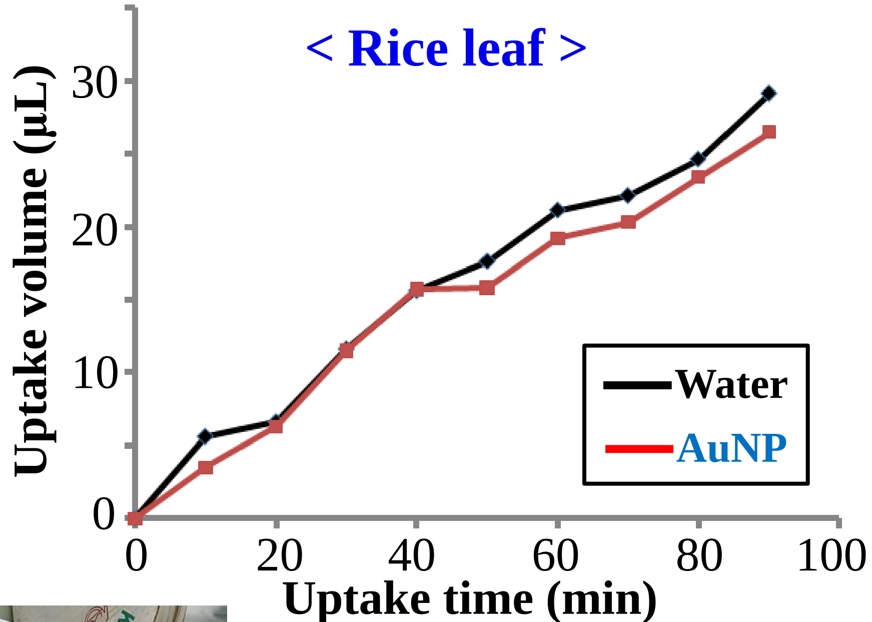


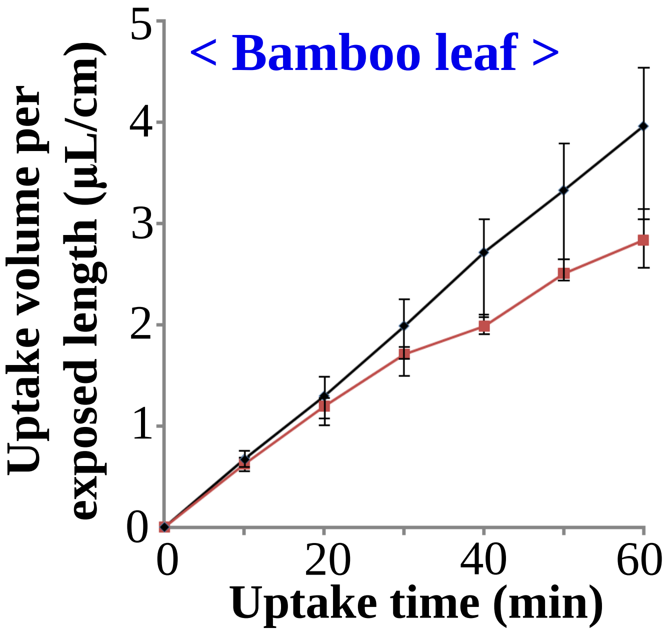
Water-refilling stage ⇒ increase AuNPs staining

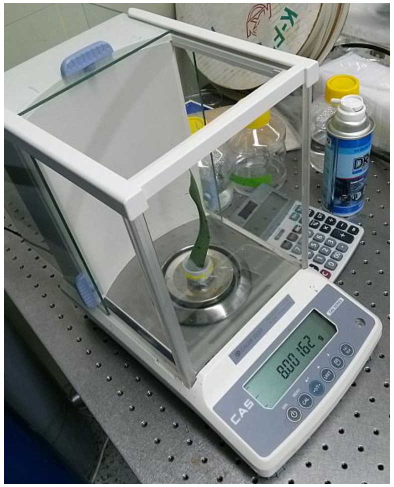
Ratio of the water-stoppage time at perforation plates to the total time elapsed for water-refilling in xylem vessels of a maize leaf.

- **Average contribution is stably maintained at 29% (variation: +13 ~ -19%)**
  - → **The supply of AuNPs does not induce drastic change in the functions of perforation plates in the sap transport in xylem vessels**
  - → **The clogging of AuNPs at perforation plates does not obstruct significantly the water-refilling process in xylem vessels**

Evaporative loss of AuNP solution and distilled water





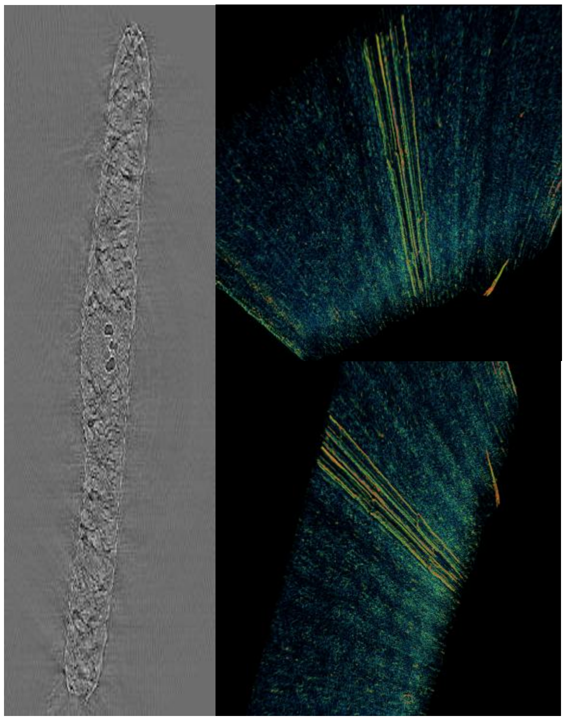


Experimental set-up

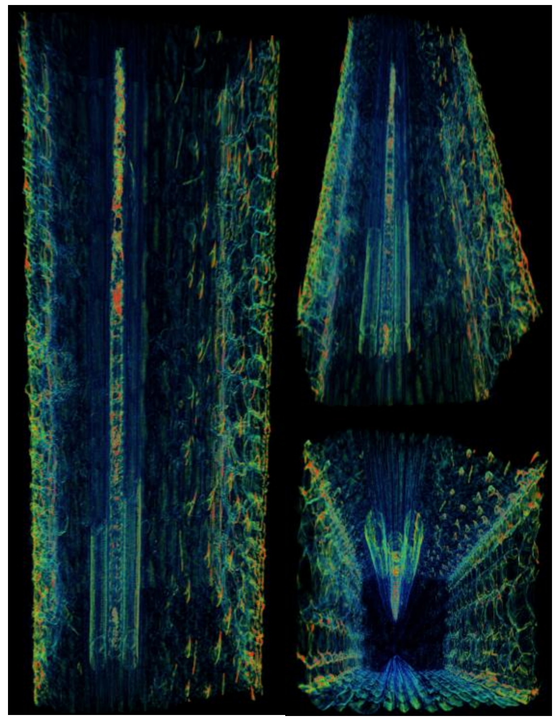
- The amounts of sap transport through xylem vessels (≡ evaporative loss of water) are compared for AuNP solution and distilled water.
- Deviation in the water-transport function starts to appear at about 20 ~ 40 min later
- The main function of xylem networks is maintained at least for the initial 20~40min.

X-ray CT imaging of 3D vascular structure using AuNPs

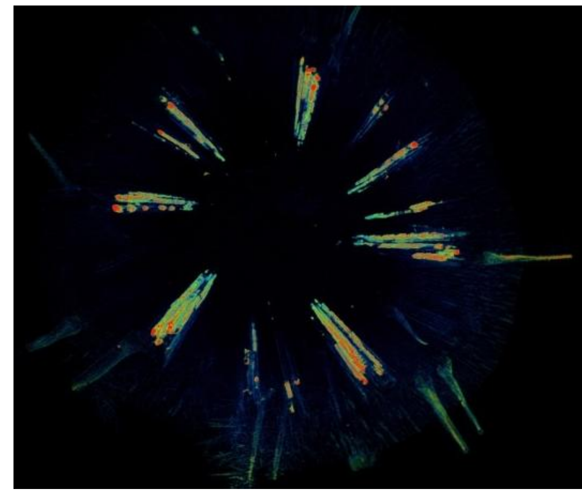
- Hydrophilic AuNPs to visualize the AuNP stained xylem vessels
- Comparison of 3D vascular structure of various plants



Rice leaf blade

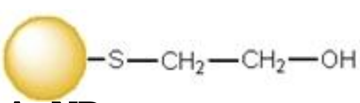


Maize leaf sheath



Arabidopsis (dicot)

Vascular bundle of monocot leaves

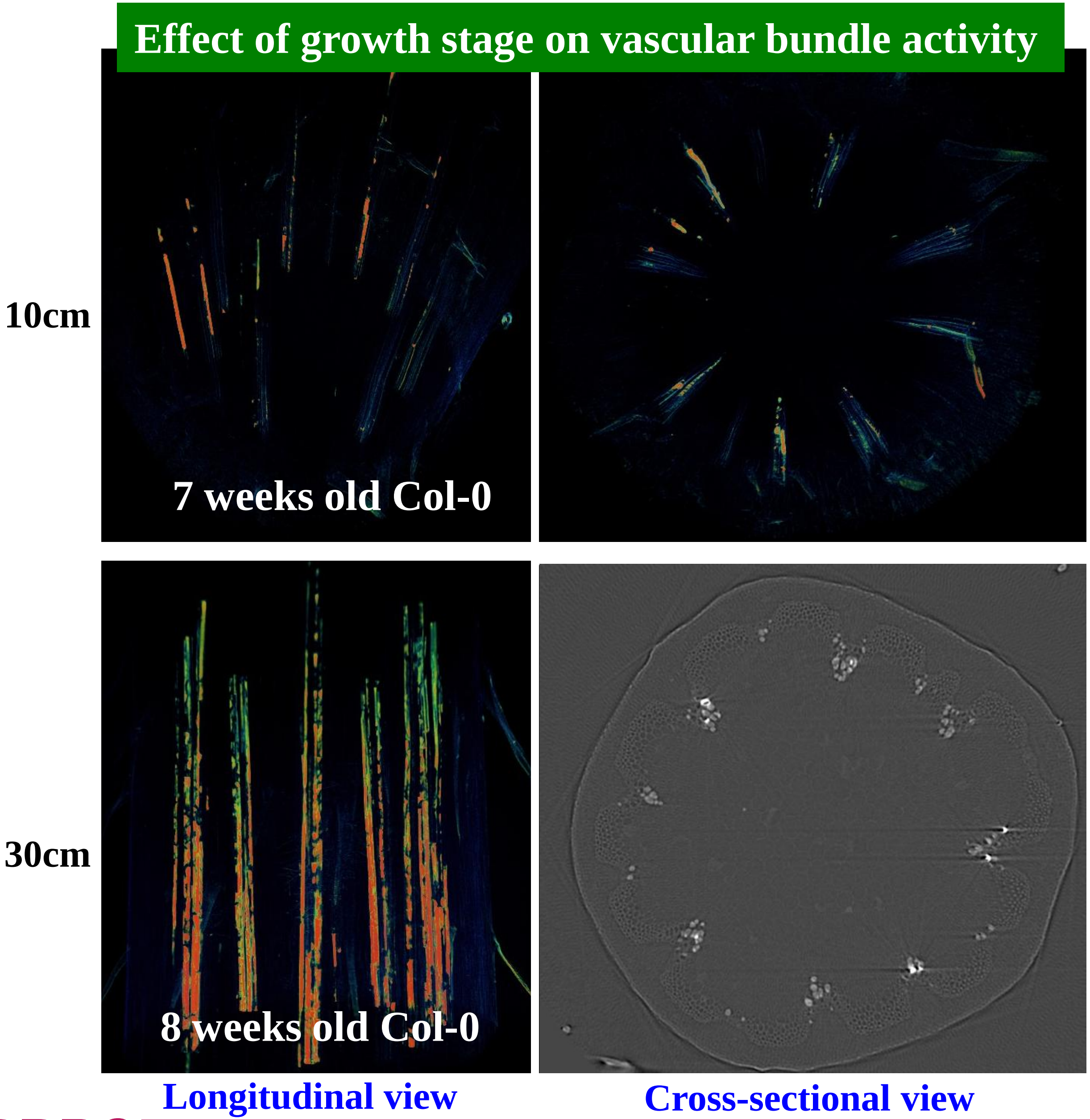


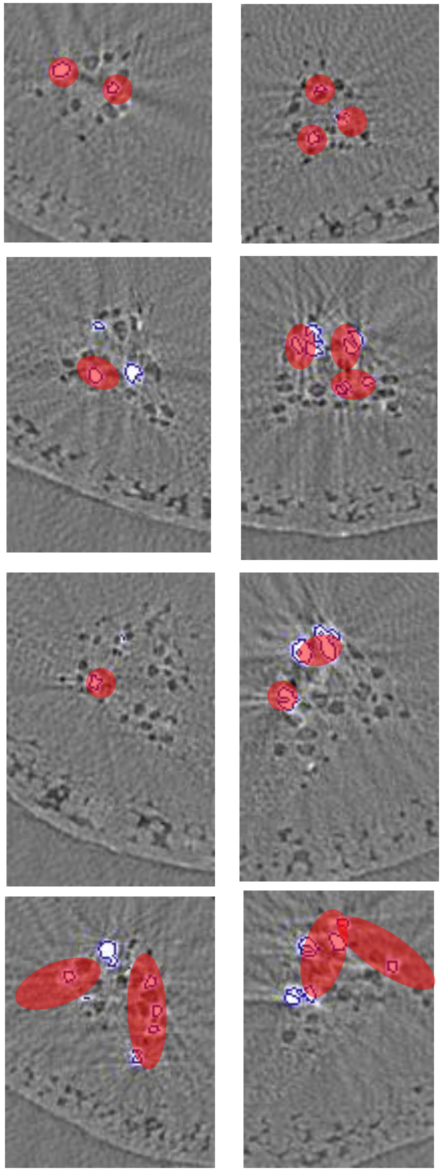
AuNP

Hydrophilic AuNP 2-mercaptoethanol
($SH\text{-}CH_2CH_2OH$)

SS Ahn et al., ACS Nano, 4, 3753-3762, 2010
HK Kim & SJ Lee, New Phytologist, 188, 1085-1098, 2010

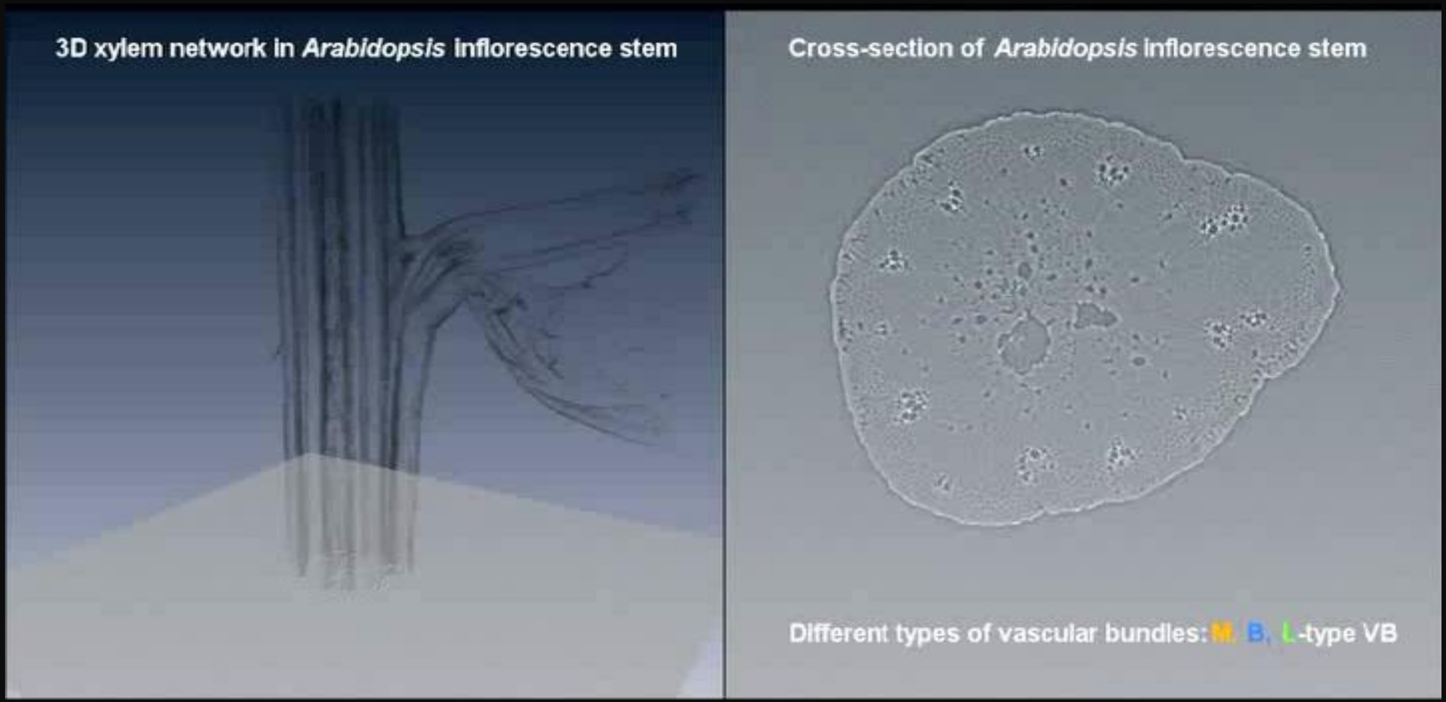
Uptake demand and vacular-bundle activity variation





3D xylem network and its changes along the height

3D xylem network in *Arabidopsis* **Cross-sectional view**

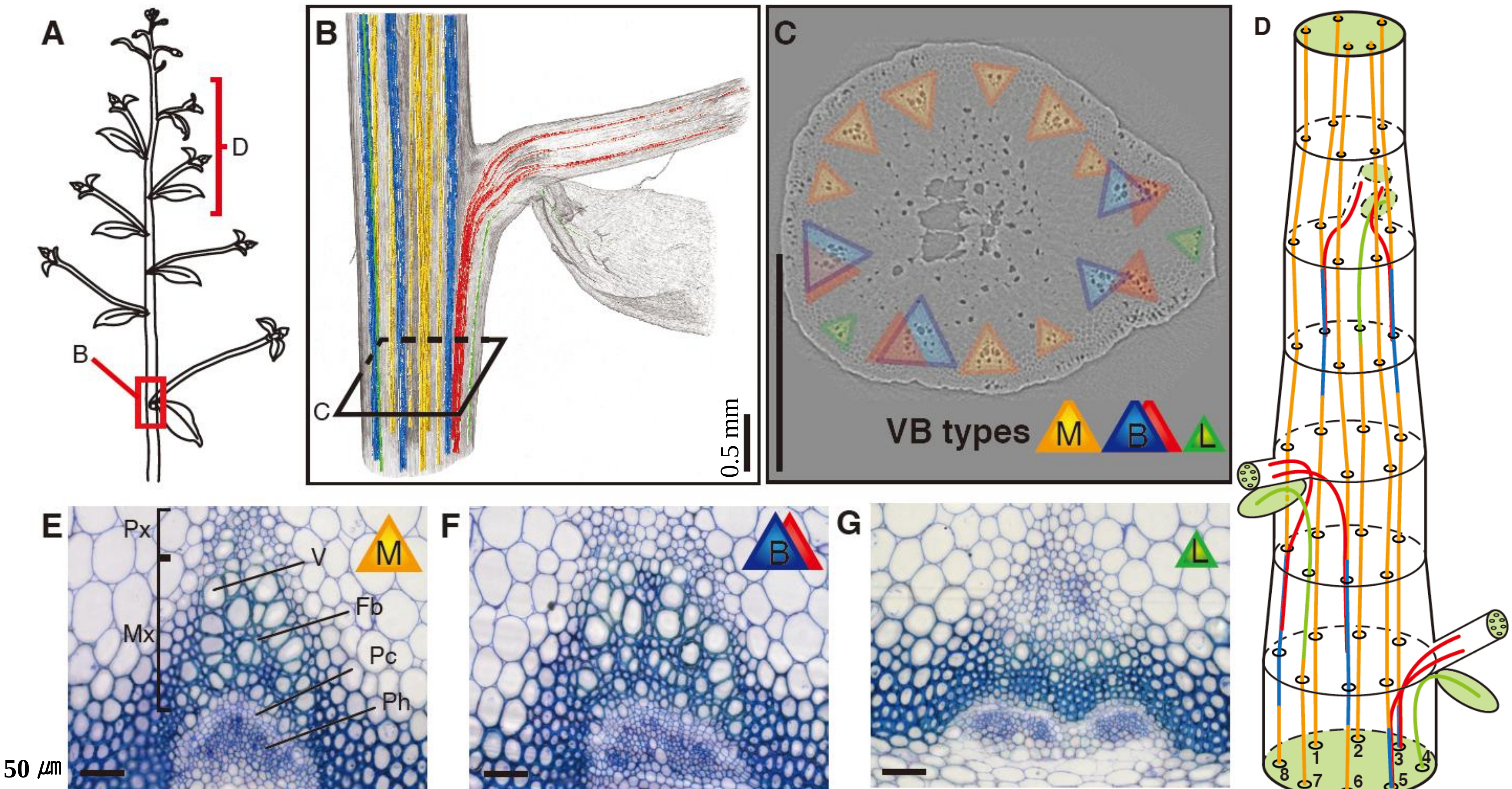


Different type of VBs : M (main stem), B (branching), L (cauline leaf)

VB types are changing according to the development stage and SXRCT is useful for observing the reorganization of VBs.

Xylem network & classification of VB types

Xylem structures visualized by Sync X-ray CT are classified into three VB types (M, B, L) having unique properties at a given height.

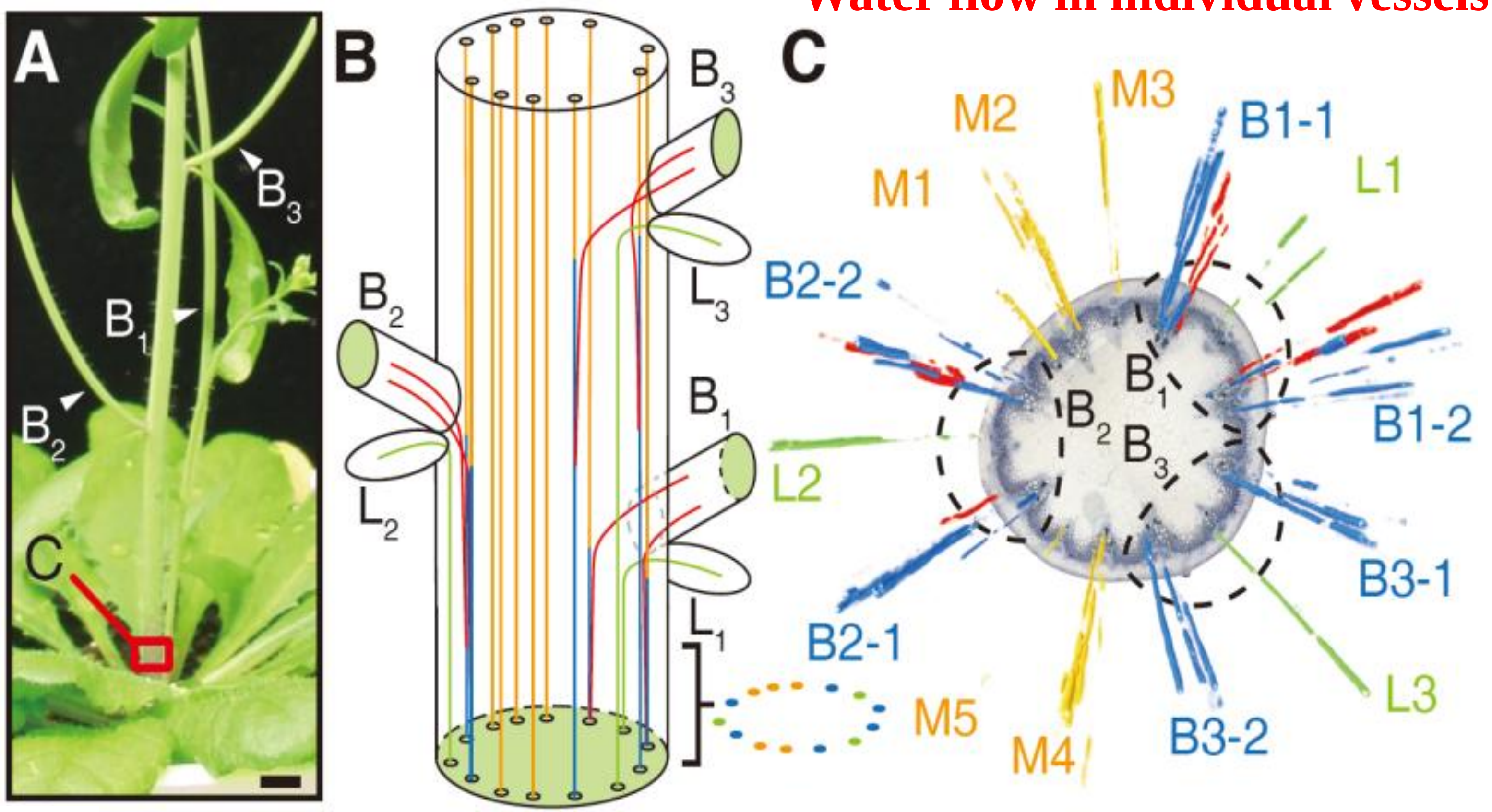


M (main stem) **B (branching)** **L (cauline leaf)**

Px: protoxylem, Mx: metaxylem, Fb: fiber, Pc: procambium, Ph: Phloem, V: vessel

Water flow visualized by AuNPs staining

Water flow in individual vessels



Not all vessels are functionally equivalent, rather regulated by connected tissues.

Summary

- X-ray micro-imaging technique is useful and unique for visualizing water transport in xylem vessels of vascular plants with high spatial and temporal resolution.
- X-ray CT combined with hydrophilic AuNPs can be used to reveal the complex xylem networks and water transport in xylem vessels.

Thank you for kind attention