Cytomechanic Regulation of Pollen Tube Growth

MecanX

MecanX – Understanding Physics of Plant Growth

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Amphacademy, Root, September 15th 2017

Cytomechanic Regulation of Pollen Tube Growth

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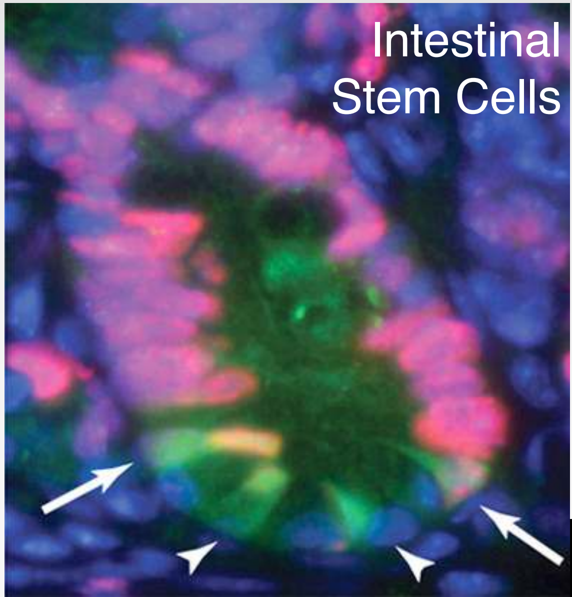
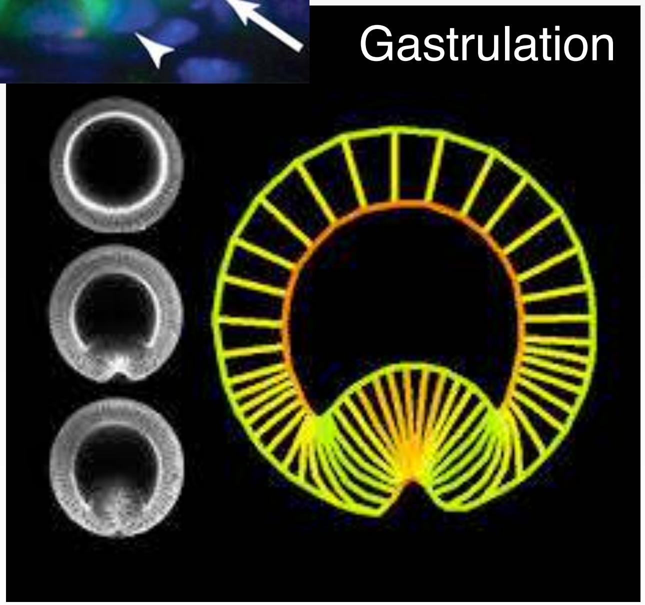
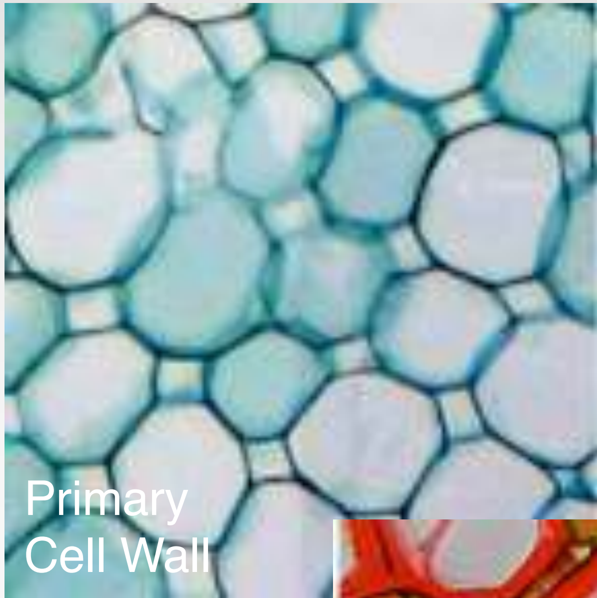
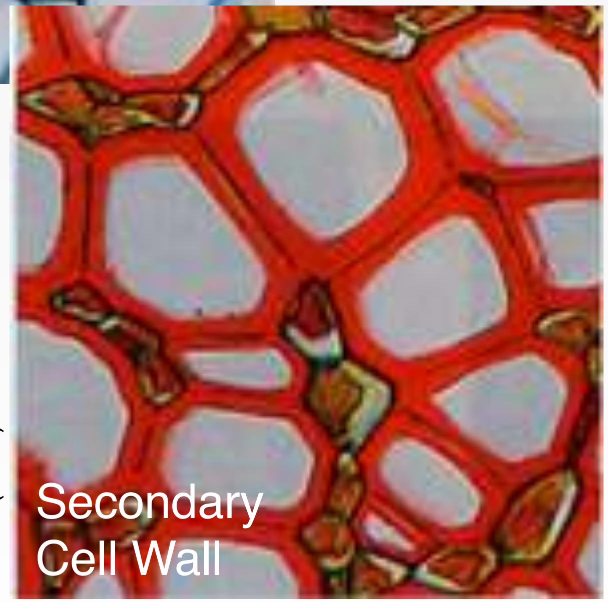
Mechanical Forces Play an Important Role in Morphogenesis





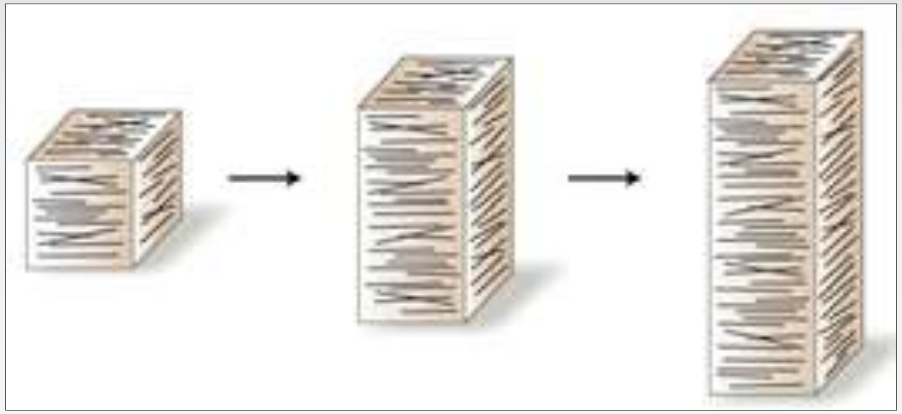
Broadland et al. (2010) *PNAS* 107, 22111
van Dussen et al. (2011) *Development* 139, 488



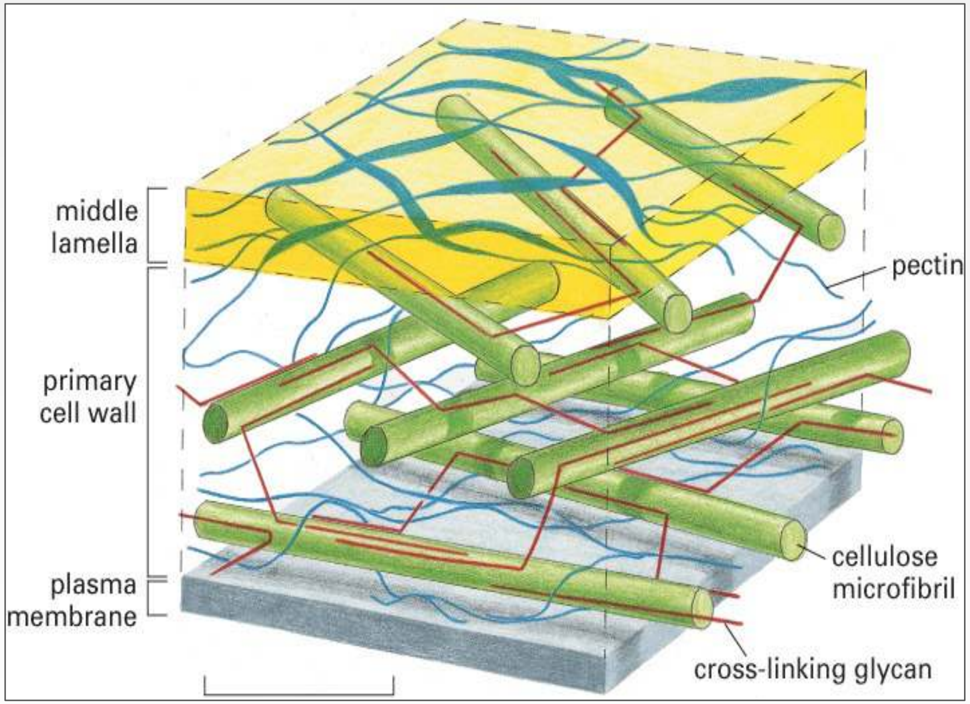


Taiz and Zeiger, *Plant Physiology* 5th Edition (2010)

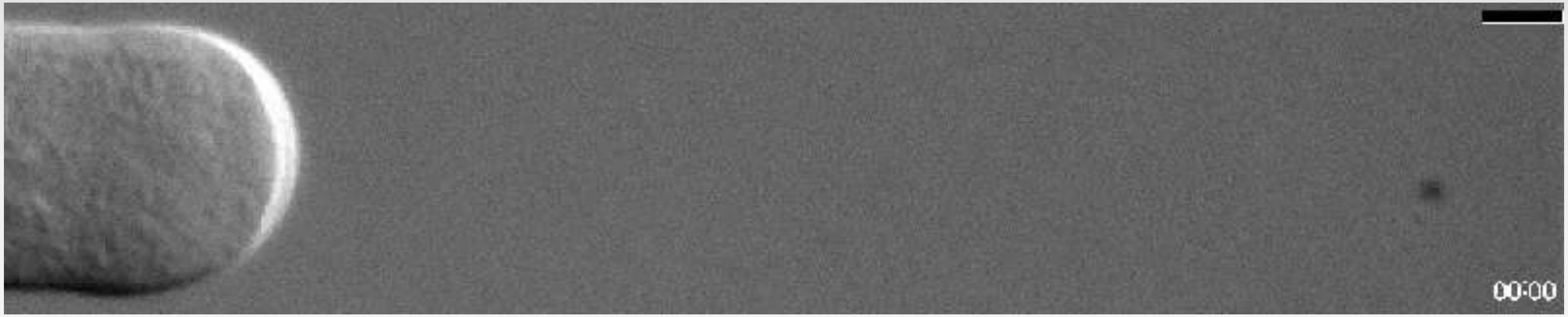
Cell Wall and Plant Growth



- cell expansion is driven by **turgor pressure**
- requires coordination between **loosening/deformation** of existing cell wall and the secretion of new cell wall material
- relationship between **biochemical** composition and **mechanical** properties of the cell wall are **not well understood**



Mechanics of Pollen Tube Growth



Movie: Peter Hepler

Goals of the **MecanX** project are:

- to develop novel methods to **measure** mechanophysical properties
- to investigate how the biochemical composition affects the **mechanical properties** of the cell wall
- to develop **mathematical models** to describe plant cell growth based on cytomechanical parameters

We use **pollen tubes** as our model system because:

- they are **single** cells (no confounding effects from neighboring cells)
- cell expansion is restricted to the **tip** (2-D system)
- grow very fast, such that mechanical changes are **exaggerated** and can be followed more easily

Mutants Affecting Cell Wall Composition of the Pollen Tube

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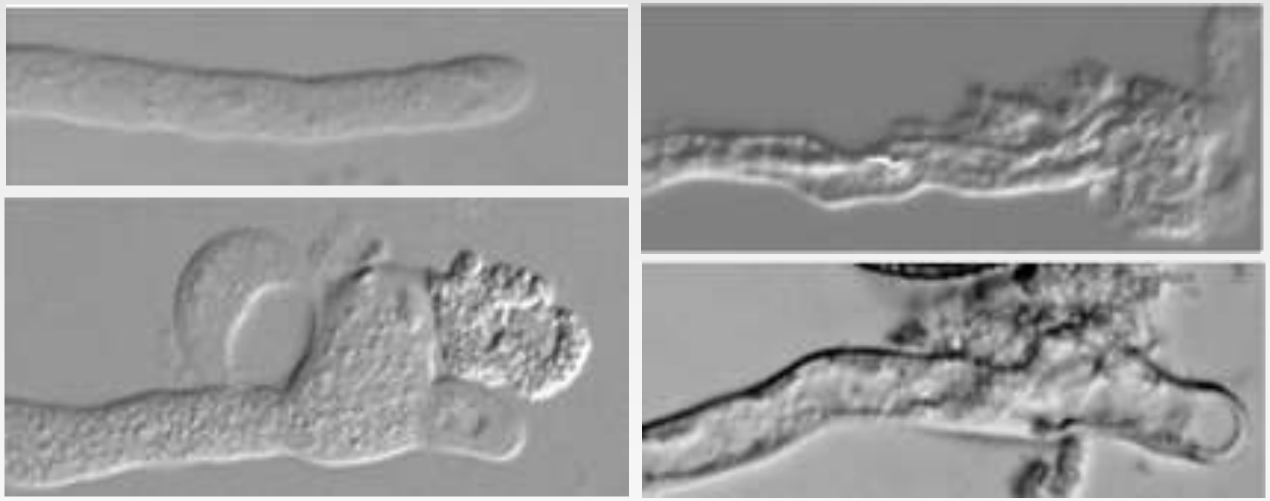
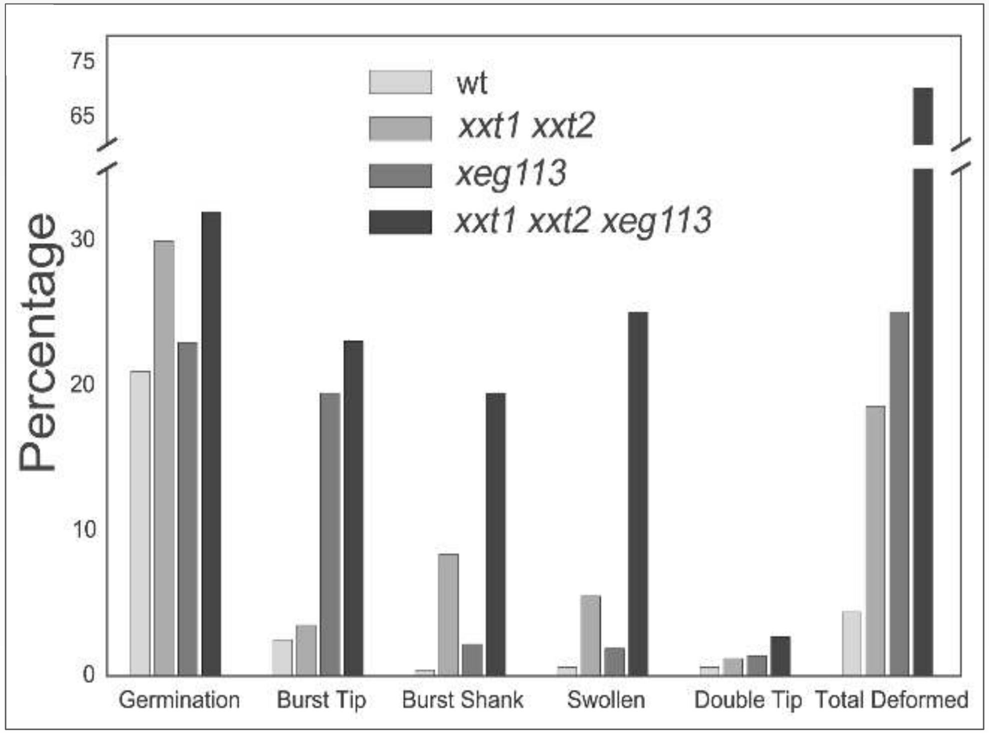
wild-type

xxt1 xxt2 (deficient in xyloglucans)

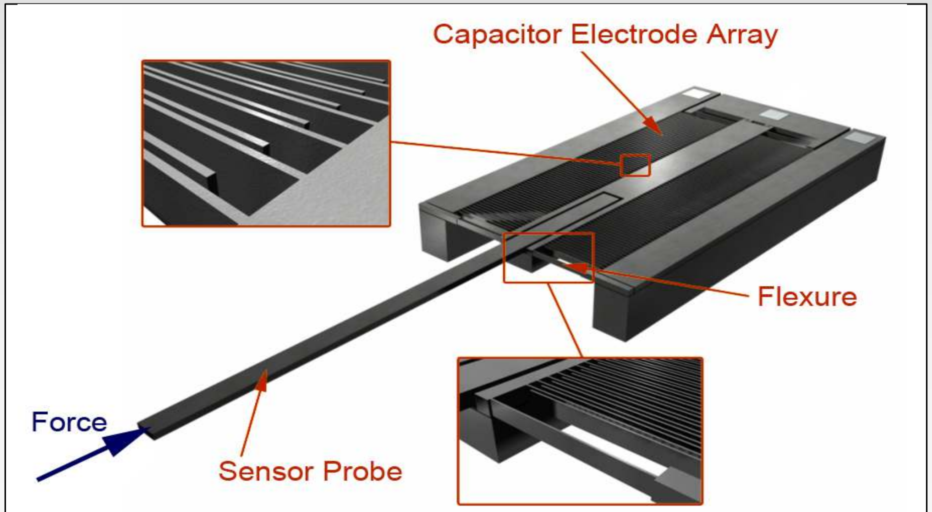


xeg113 (non-functional extensins)

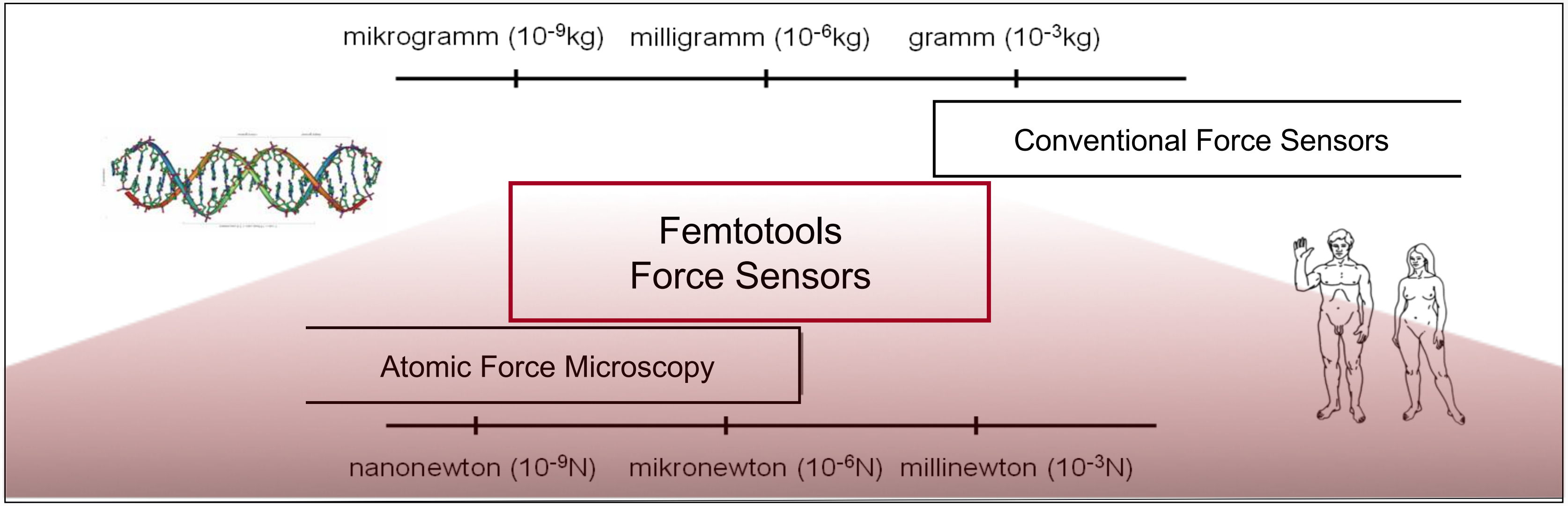
xxt1 xxt2 xeg113



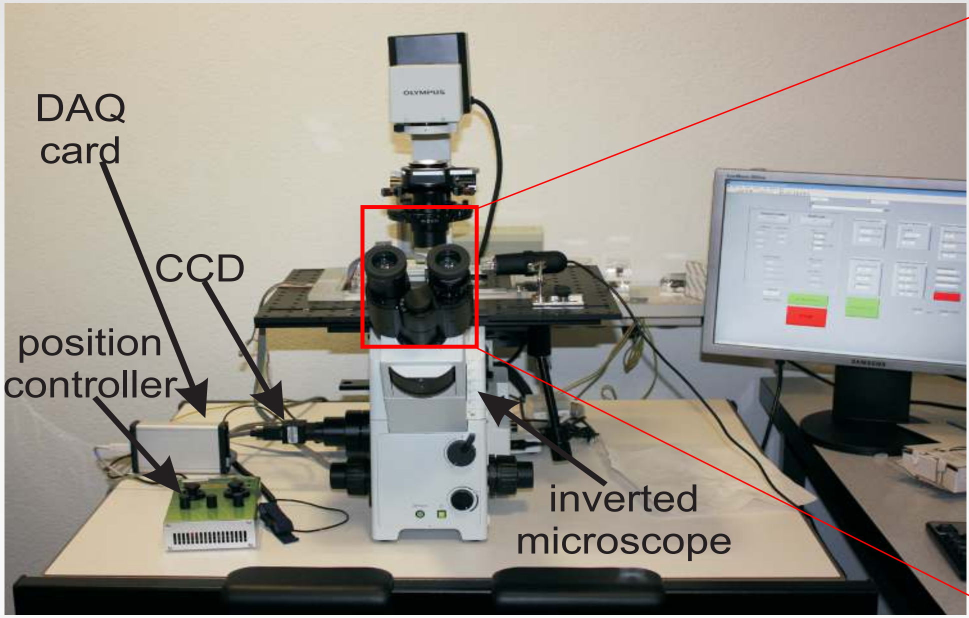
Microelectronic Mechanical Systems (MEMS)-Based Force Sensor

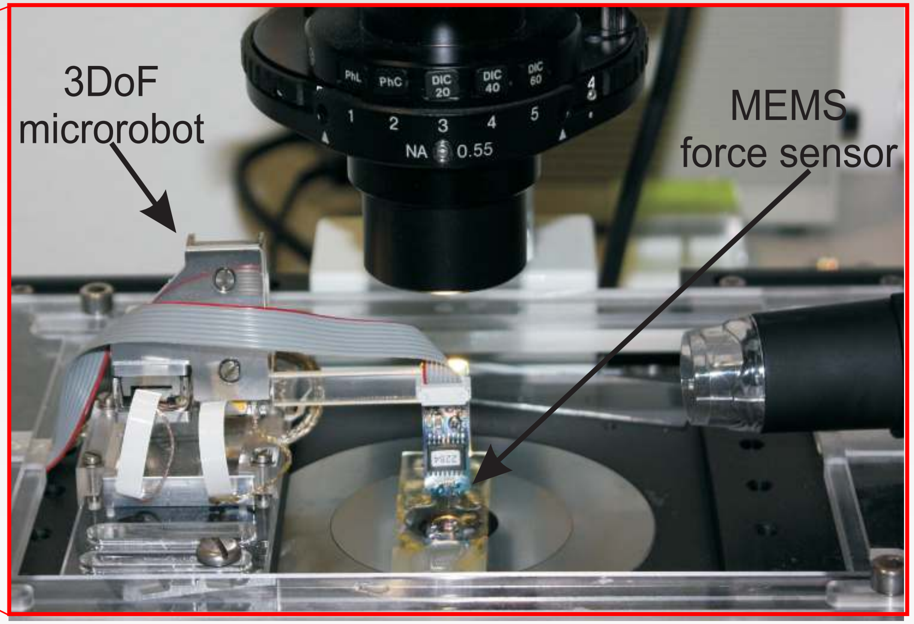


1-D MEMS-based force sensor



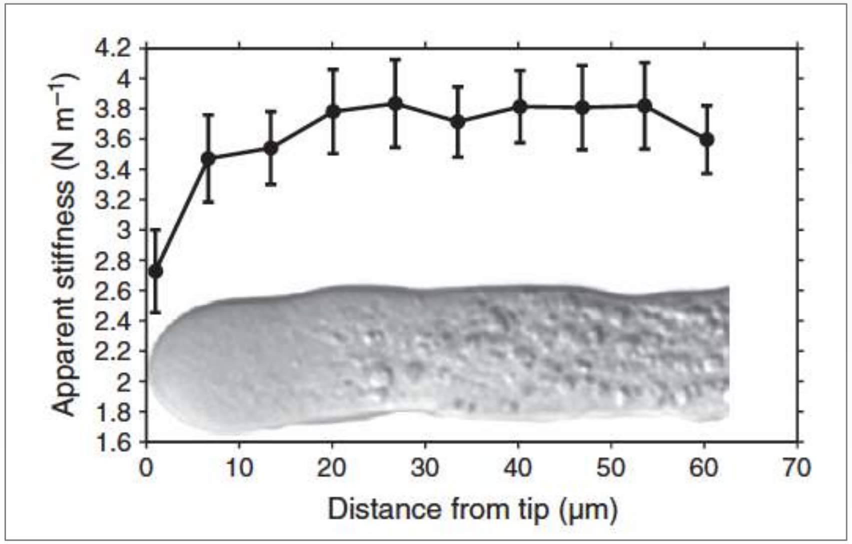
Cellular Force Microscope (CFM)





Measured **apparent stiffness** depends on:

- stiffness of the cell wall (Young's modulus and wall thickness)
- turgor pressure
- geometry of the cell and interaction with the force sensor



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Vogler et al. (2013) *Plant J.* 73, 617
Felekis et al (2011) *Micro & Nano Lett.* 6, 311

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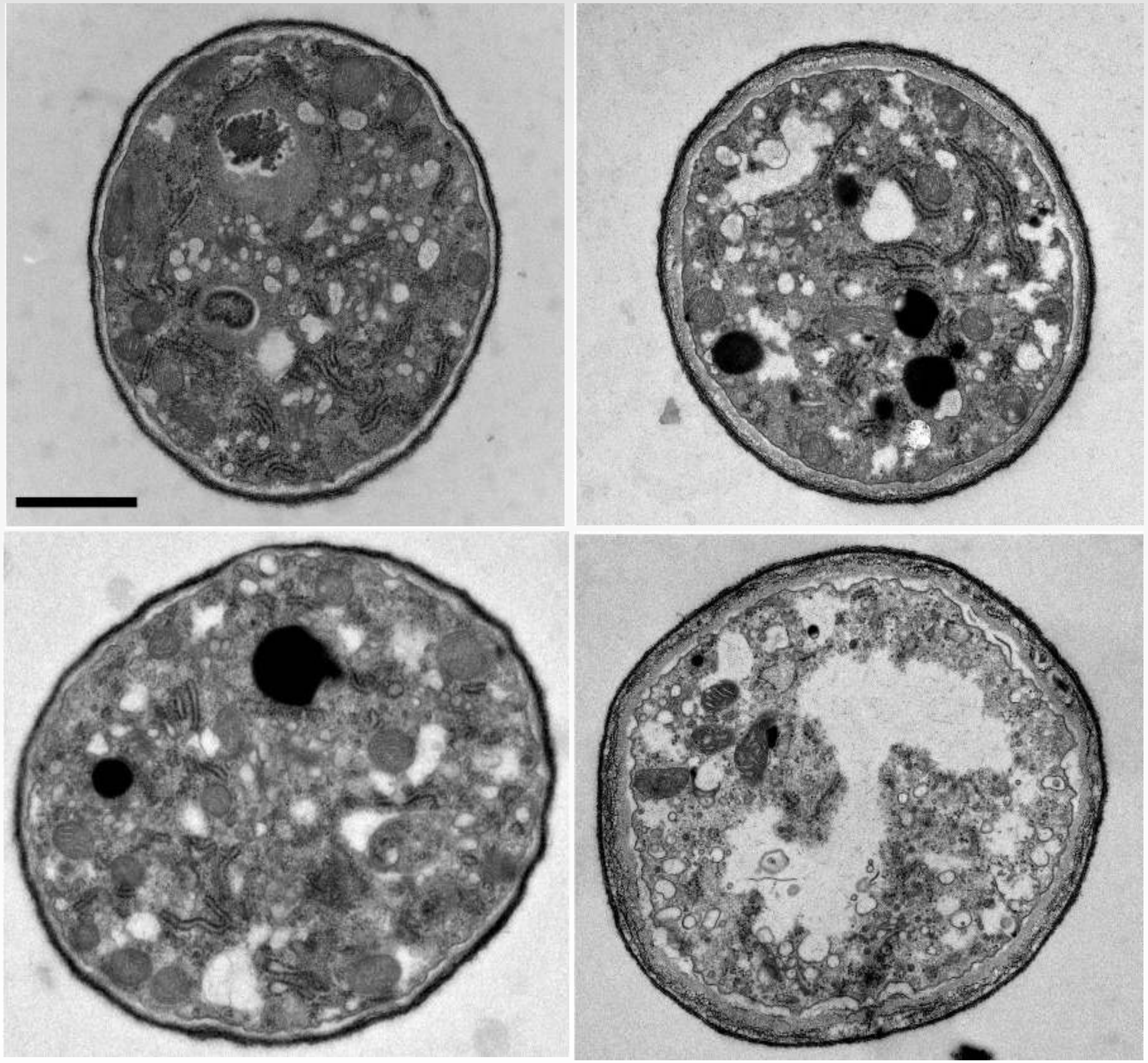
Measuring Cell Wall Thickness by TEM



wild-type

xxt1 xxt2

xeg113

xxt1 xxt2 xeg113

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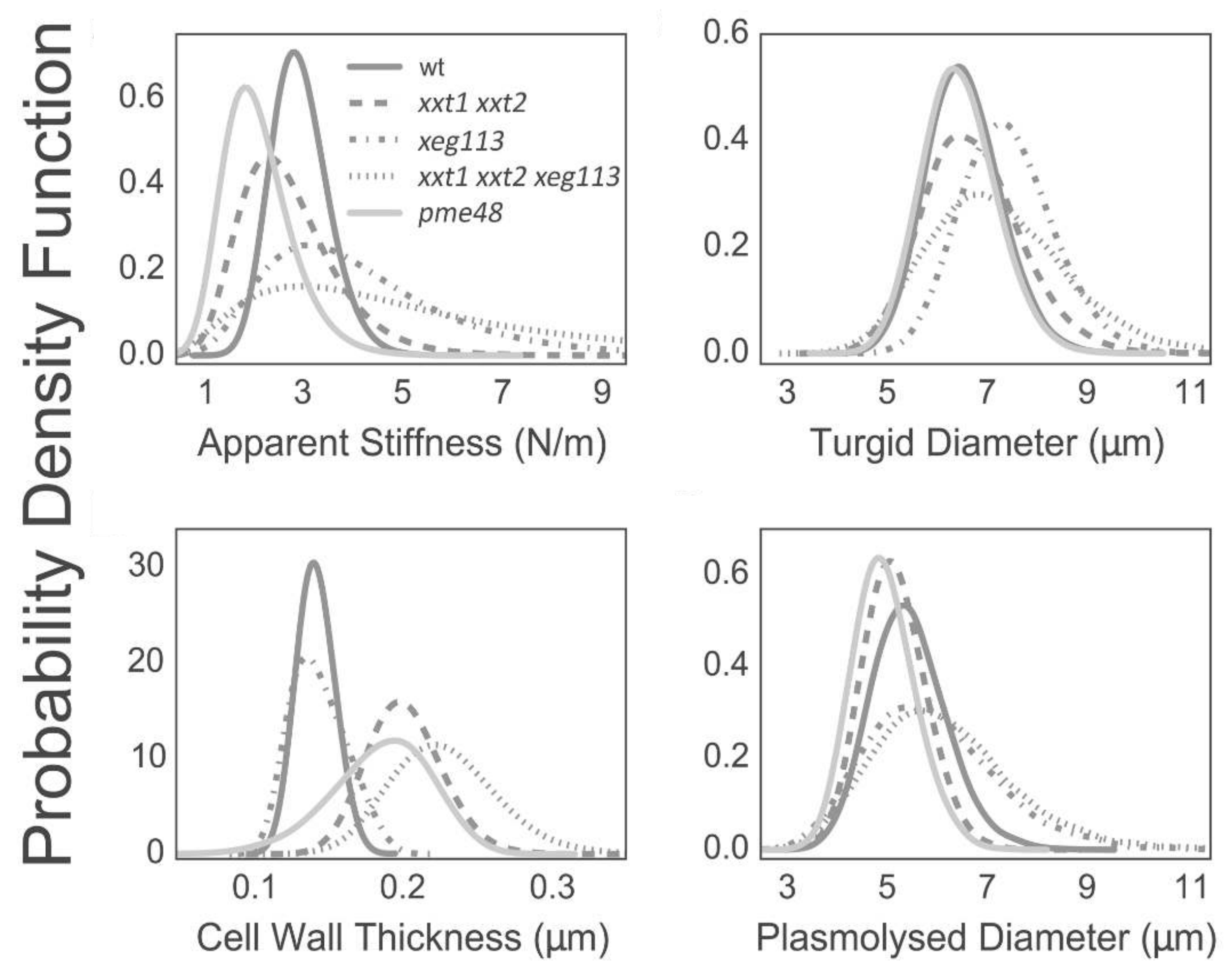
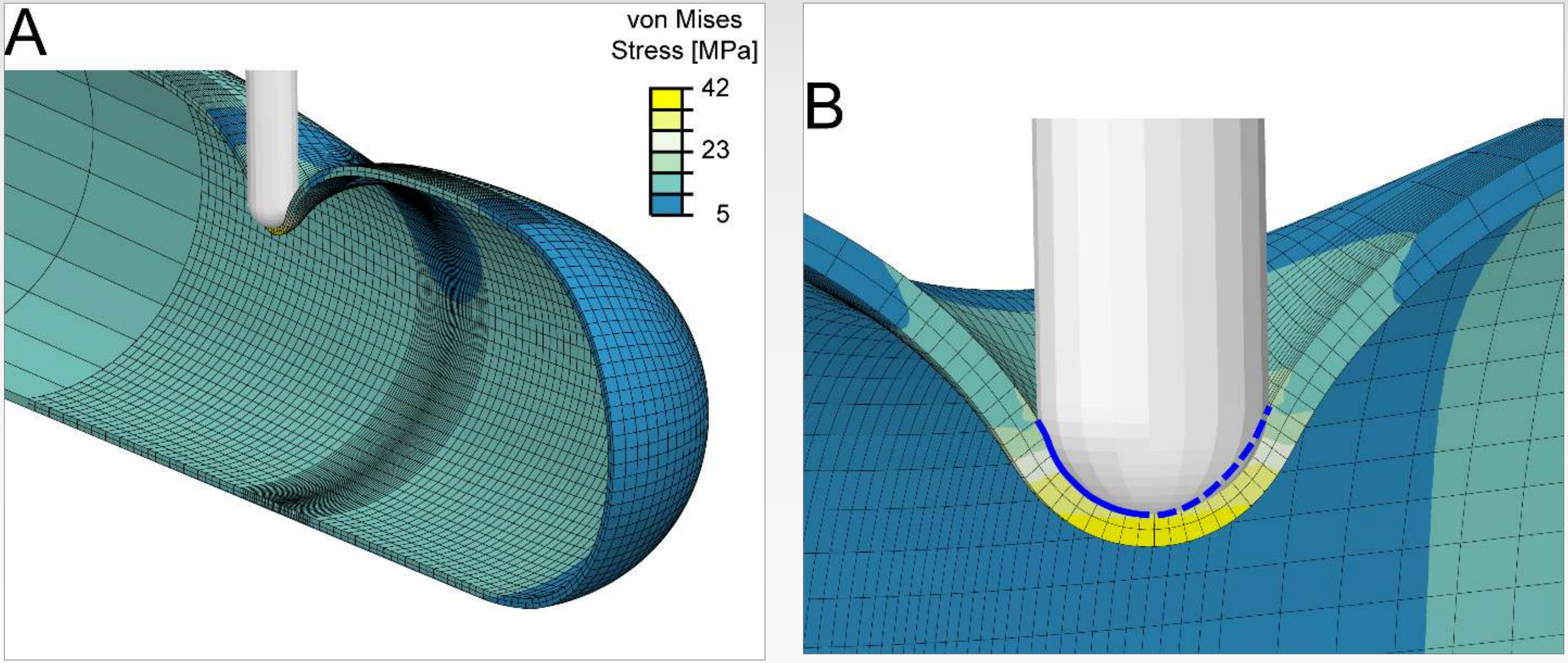
Probability Density Functions of Input Parameters for FEM Modeling



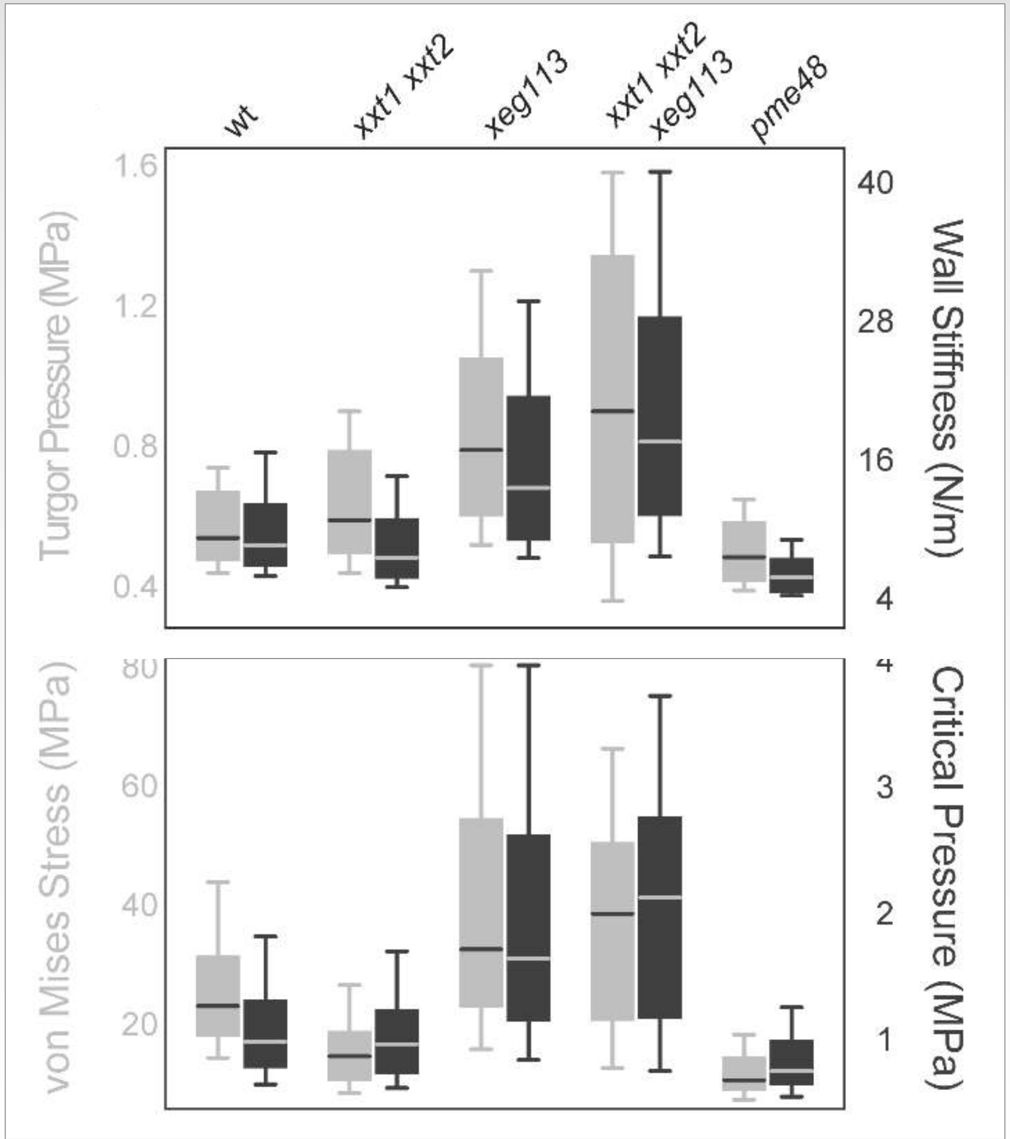
Finite Element Method-Based Modeling



Three-step FEM-based model that sequentially fits the input parameters:

- cell wall thickness (TEM)
- circumferential stretch (ratio of turgid to plasmolyzed PT diameter)
- apparent stiffness (CFM)

FEM-Based Modeling to Extract Turgor and Young's Modulus

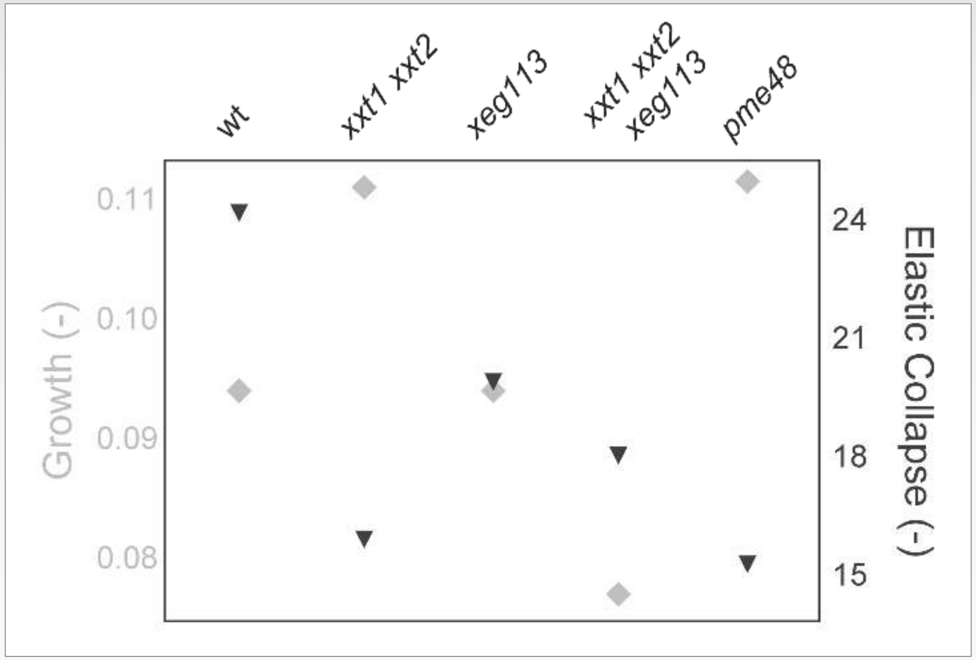


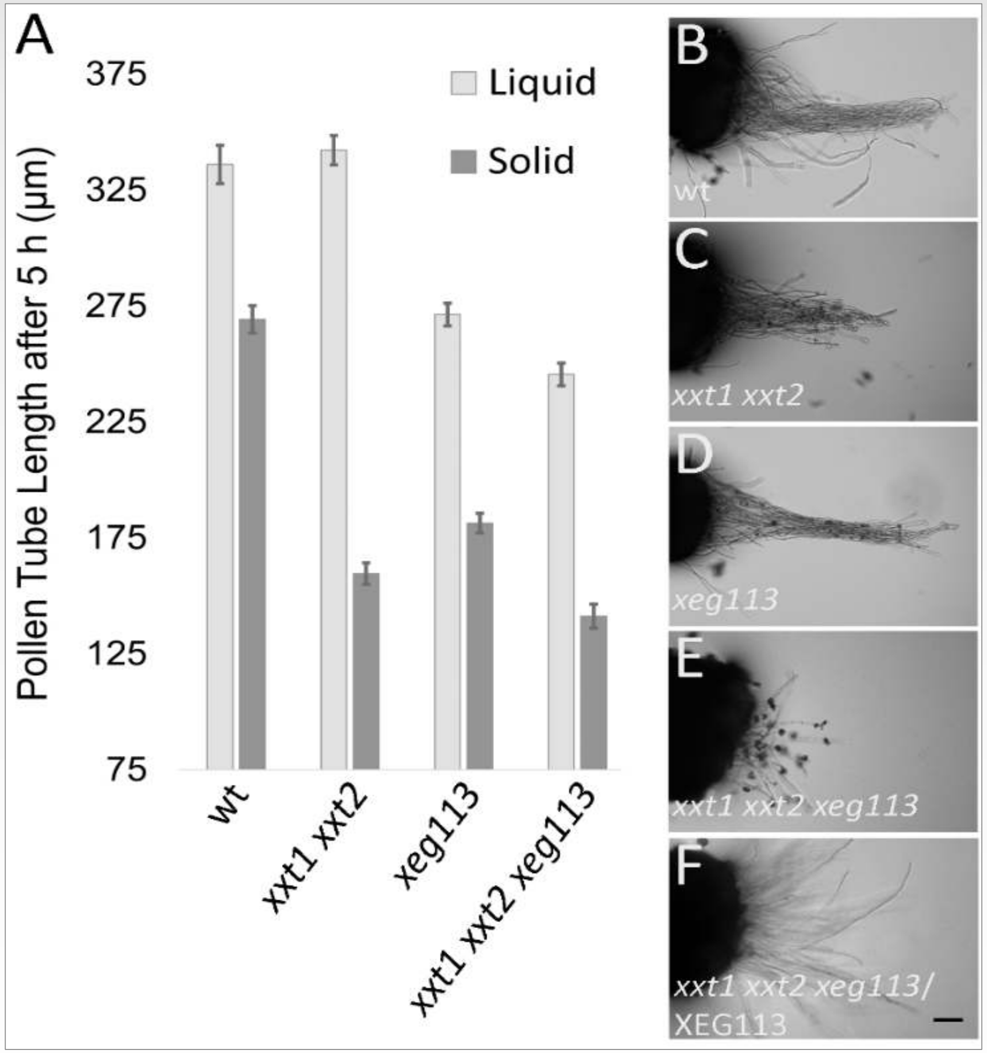
Xyloglucan-deficient PTs have a slightly elevated median turgor pressure and a reduced median cell wall stiffness.

Extensin-deficient and triple mutant PTs have a significantly increased turgor pressure and wall stiffness compared with much higher variance. Turgor and wall stiffness show a synergistic relationship.

Modeling von Mises stresses and critical pressure shows similar changes consistent with PT bursting frequencies in the mutants.

Predicted Growth Fits Observed PT Growth Behavior



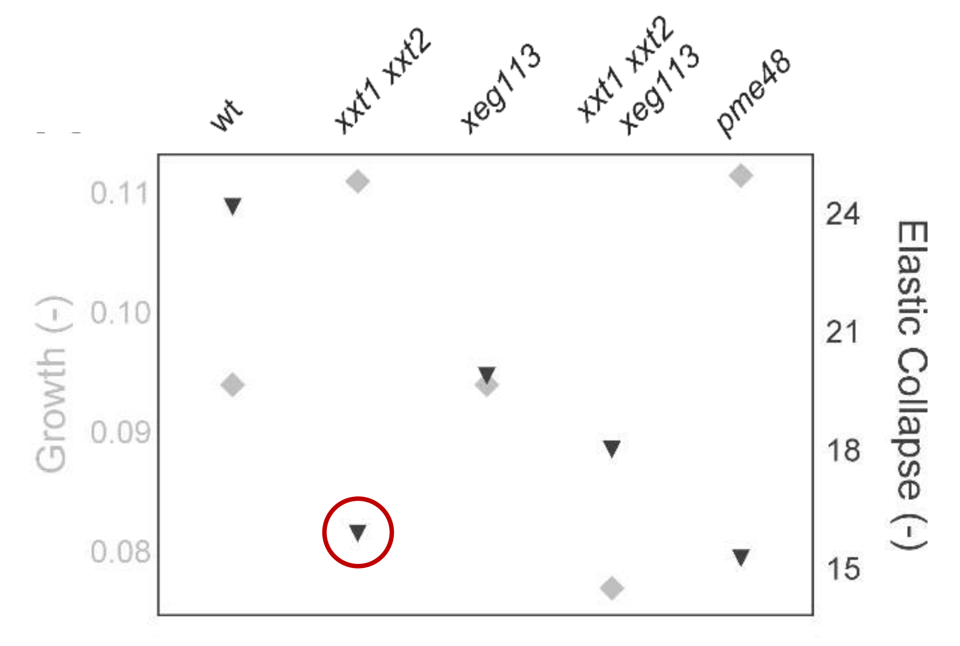


Lockhart's relation for growth in high water permeability is given by:

$$v = r_i p_i \phi$$

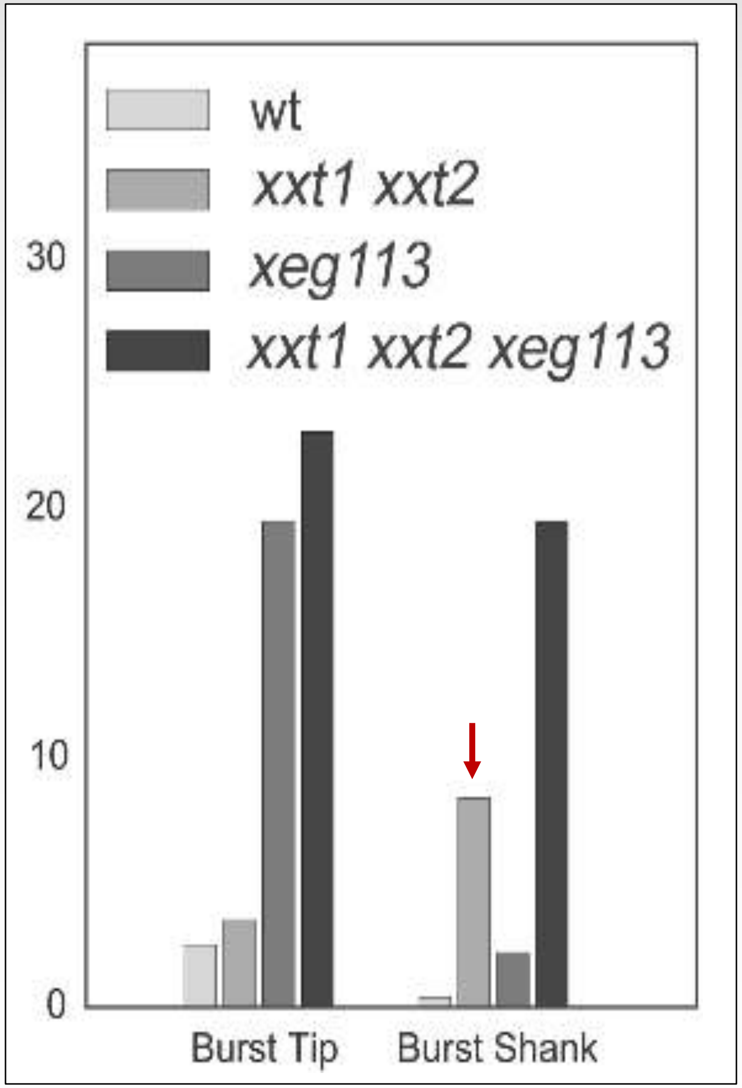
where v is the growth rate [1/hr], r_i is the radius of the cell wall not including its thickness, p_i is the turgor pressure, and Φ is the extensibility per cell wall thickness.

Shank of *xxt1 xxt2* Pollen Tubes Is Very Unstable

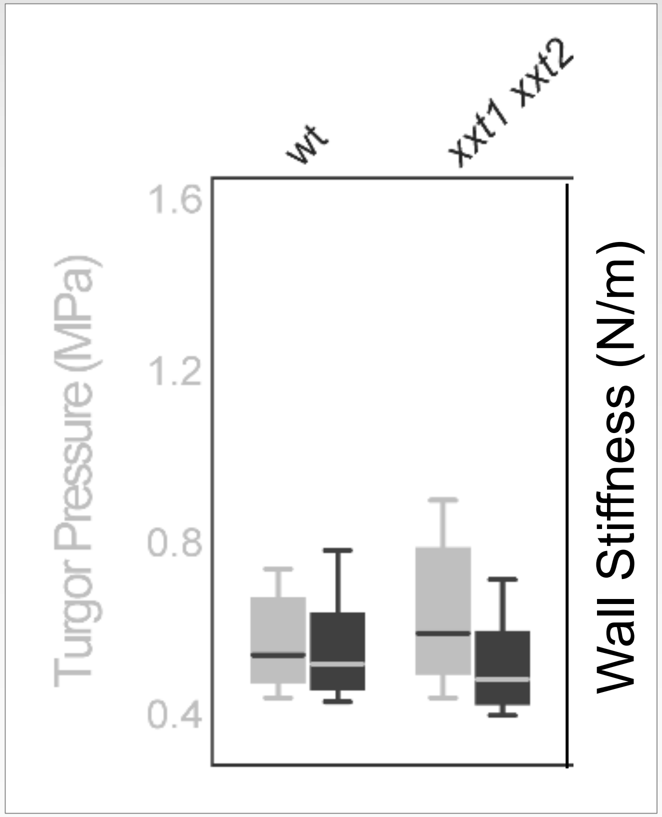


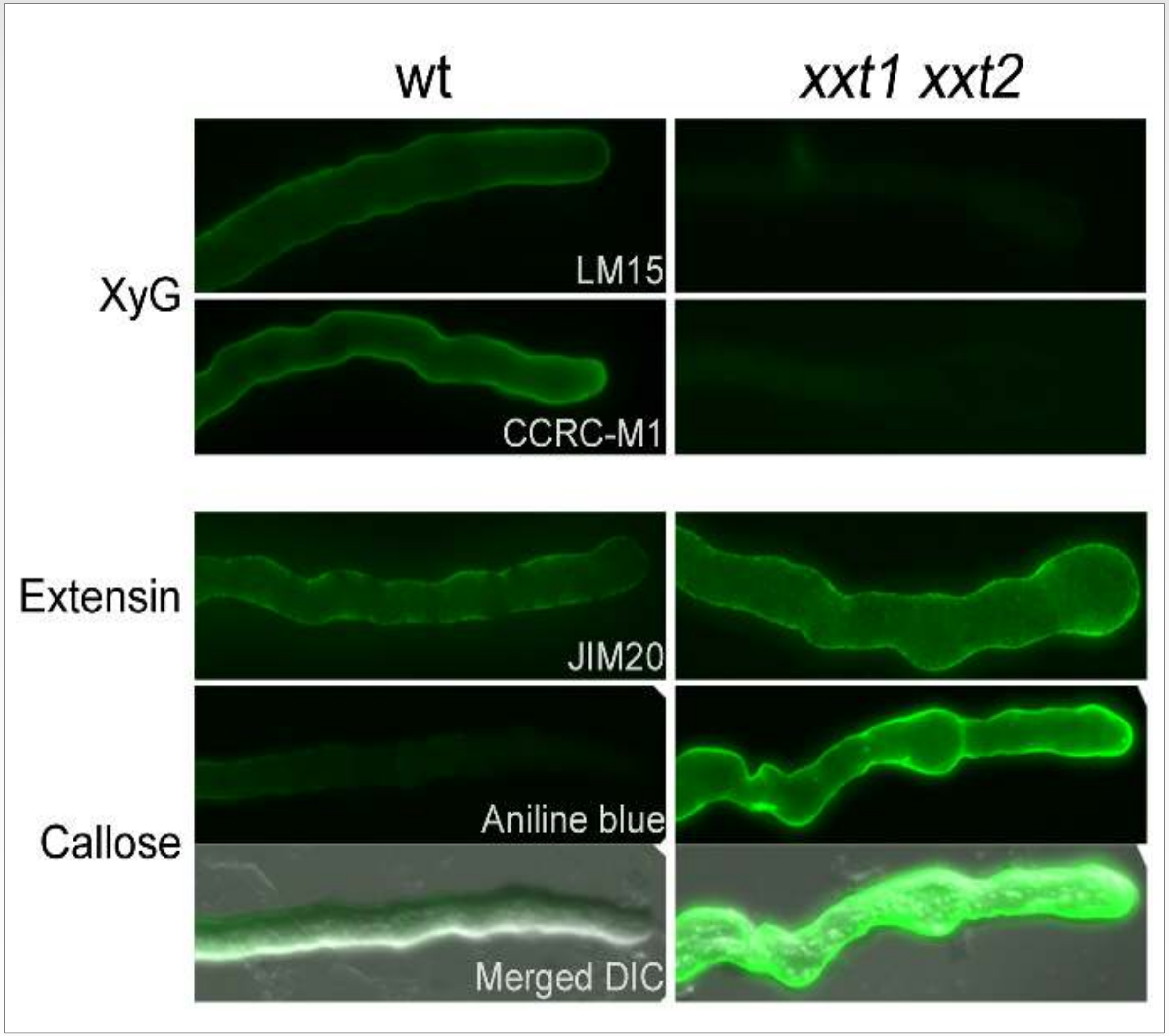
The ratio of the maximum von Mises stress to the critical pressure is a constant determining the proclivity of each mutant to become unstable and undergo an elastic collapse.

Simulations showed that the wild type has by far the most stable structure, followed by *xeg113* and *xxt1 xxt2 xeg113* mutants. The *xxt1 xxt2* PTs were the most unstable.



What Maintains the Cell Wall Stiffness in the Absence of XyG?





Compensatory changes: strongly increased callose deposition in XyG-deficient pollen tubes.

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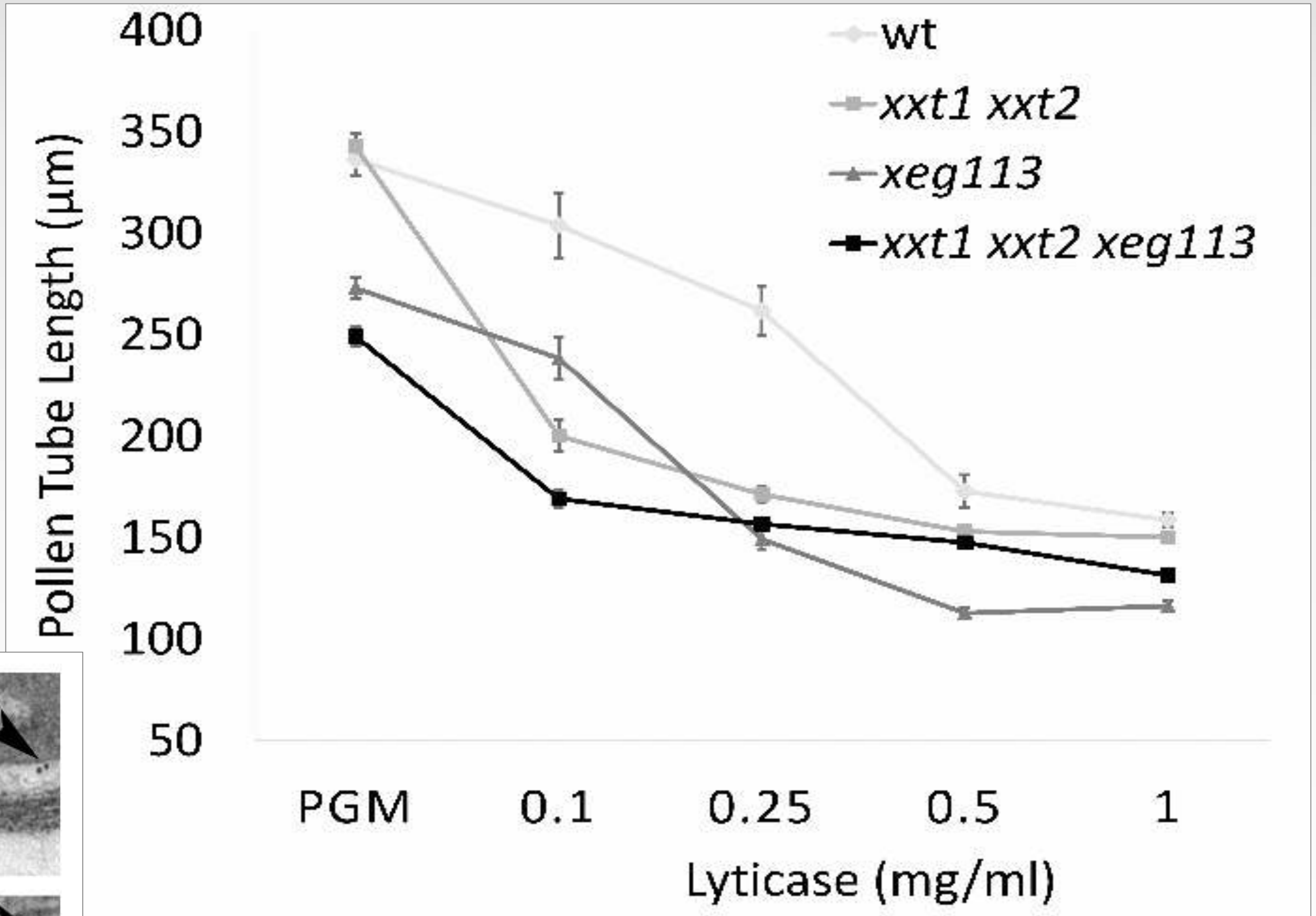
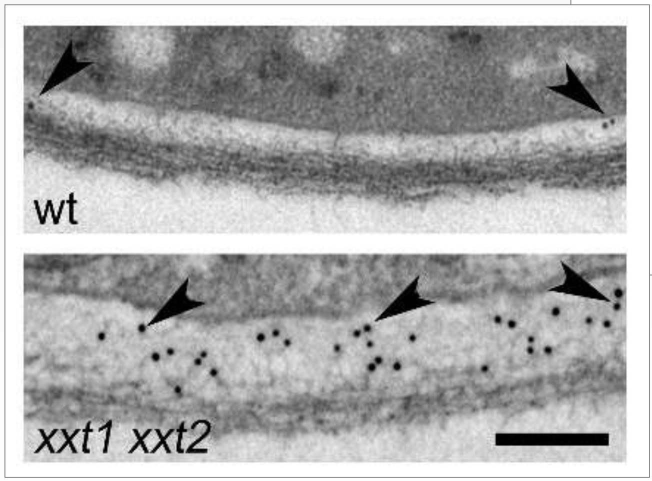
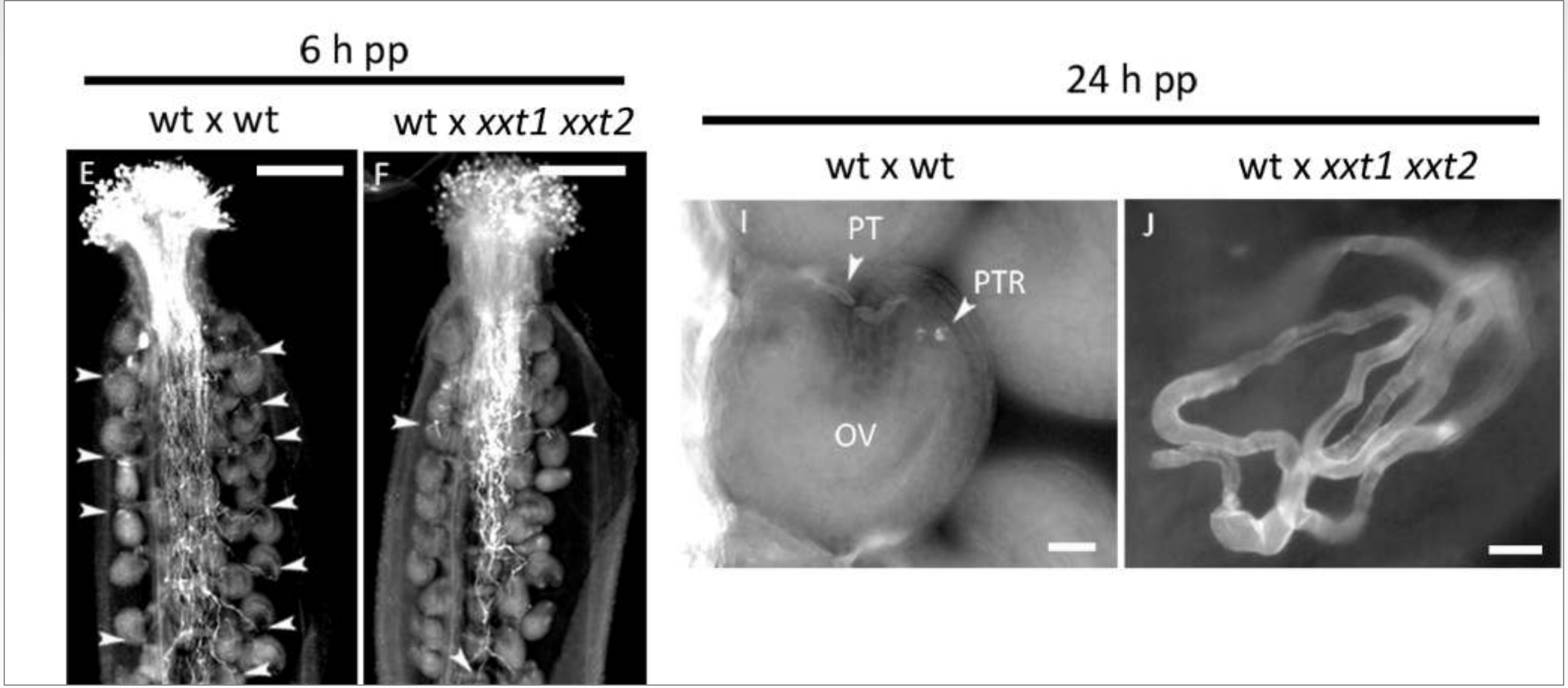
Callose-Reinforced Pollen Tubes Are More Sensitive to Lyticase



Immuno-Gold α-Callose



XyG-Deficient Pollen Tubes Grow Poorly *in vivo*



Even though *xxt1 xxt2* pollen tubes grow well *in vitro* they are poor performers *in vivo*.

Can we measure cytomechanical parameters that would approximate *in vivo* growth?

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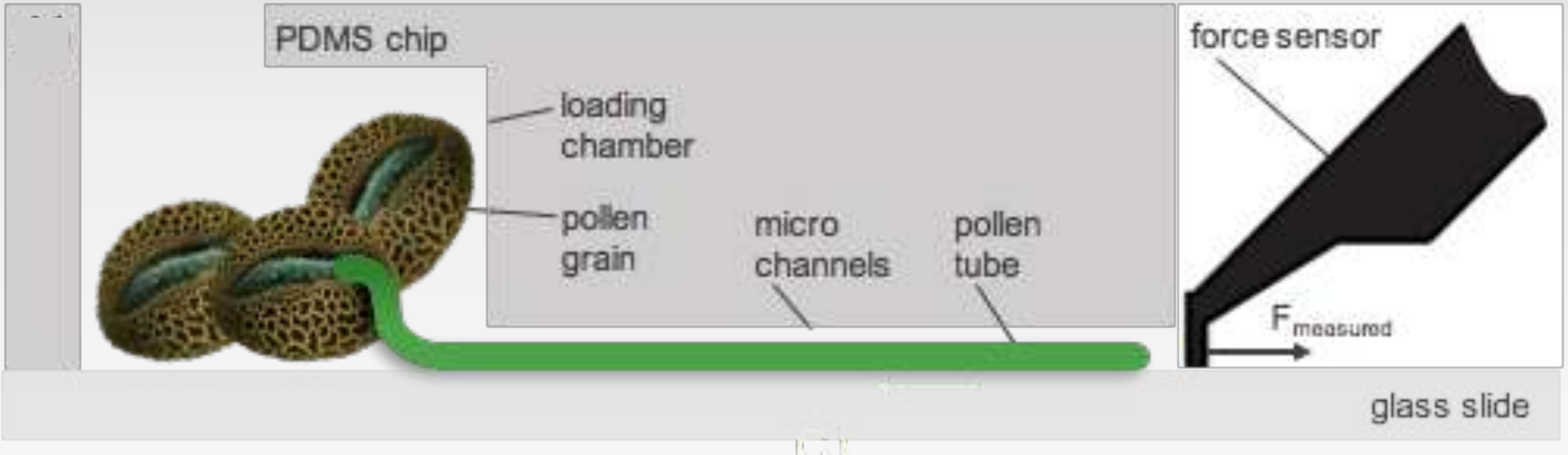
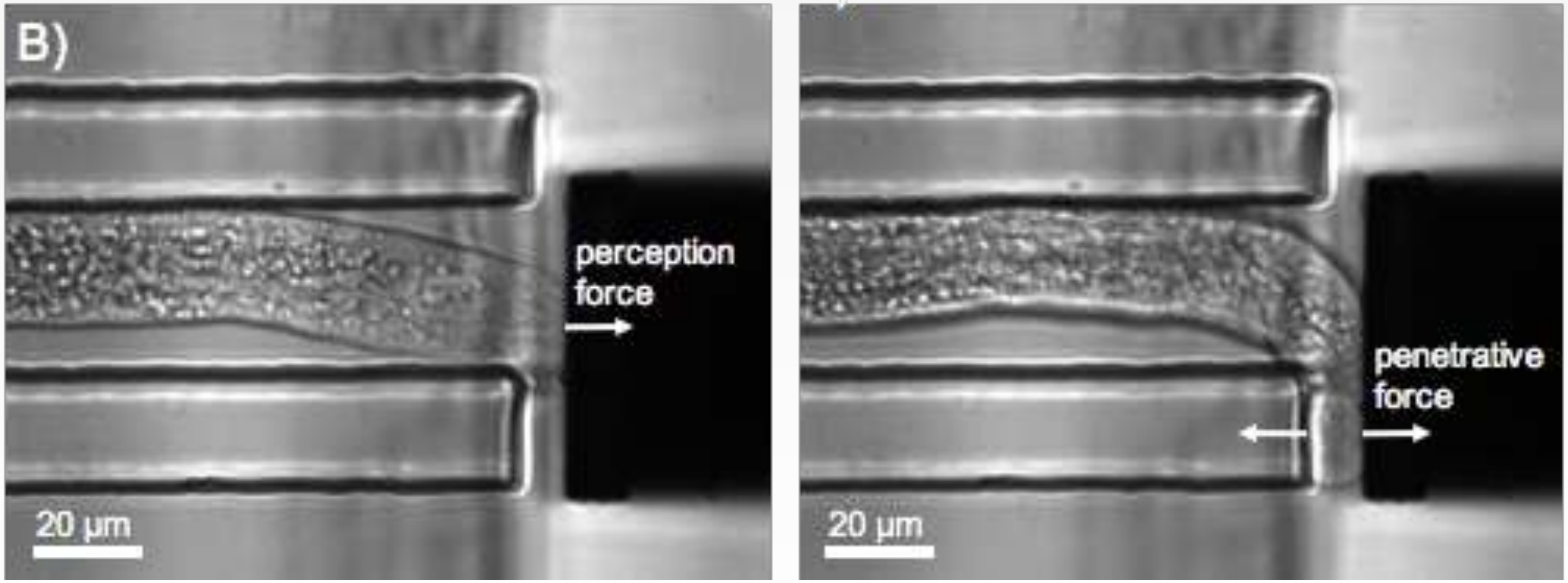
Microfluidics Chip and Lateral Force Sensor





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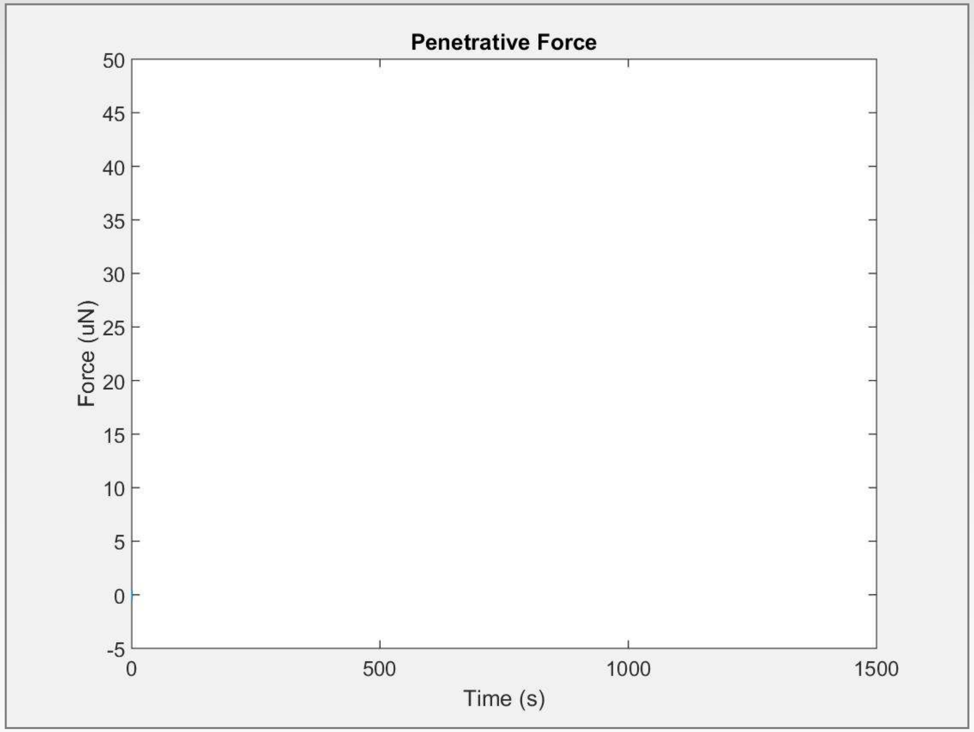
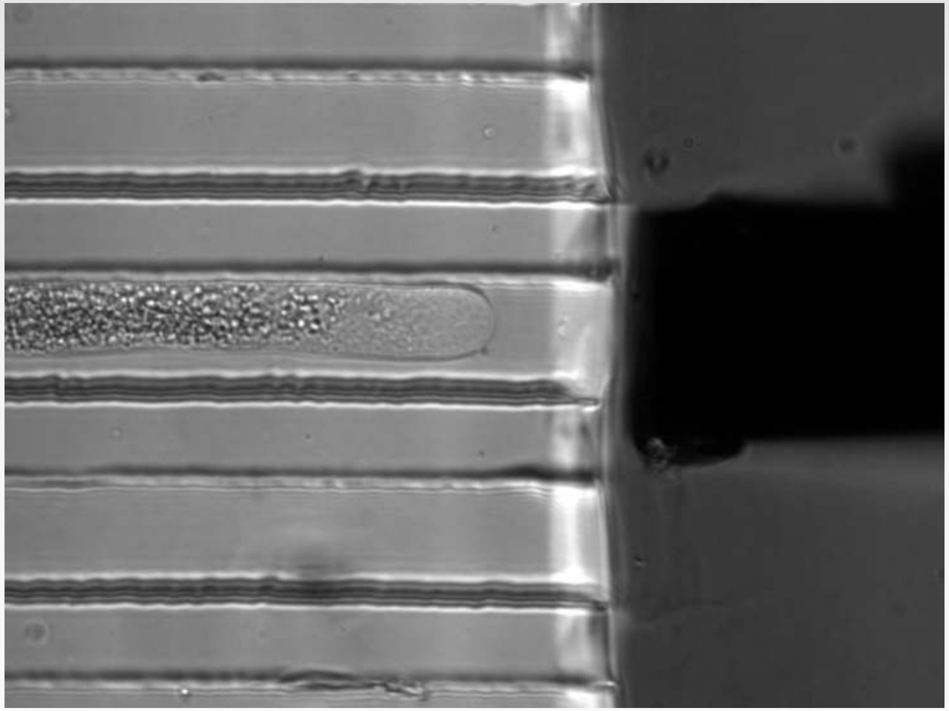
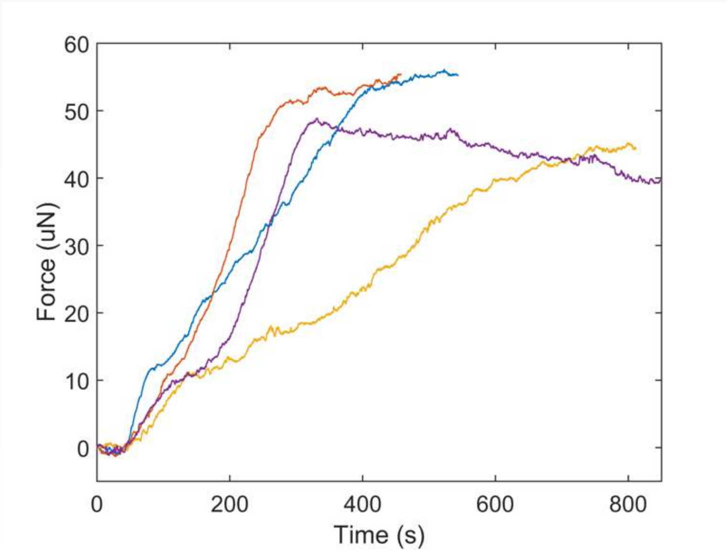
Perception and Penetrative Force Measurements







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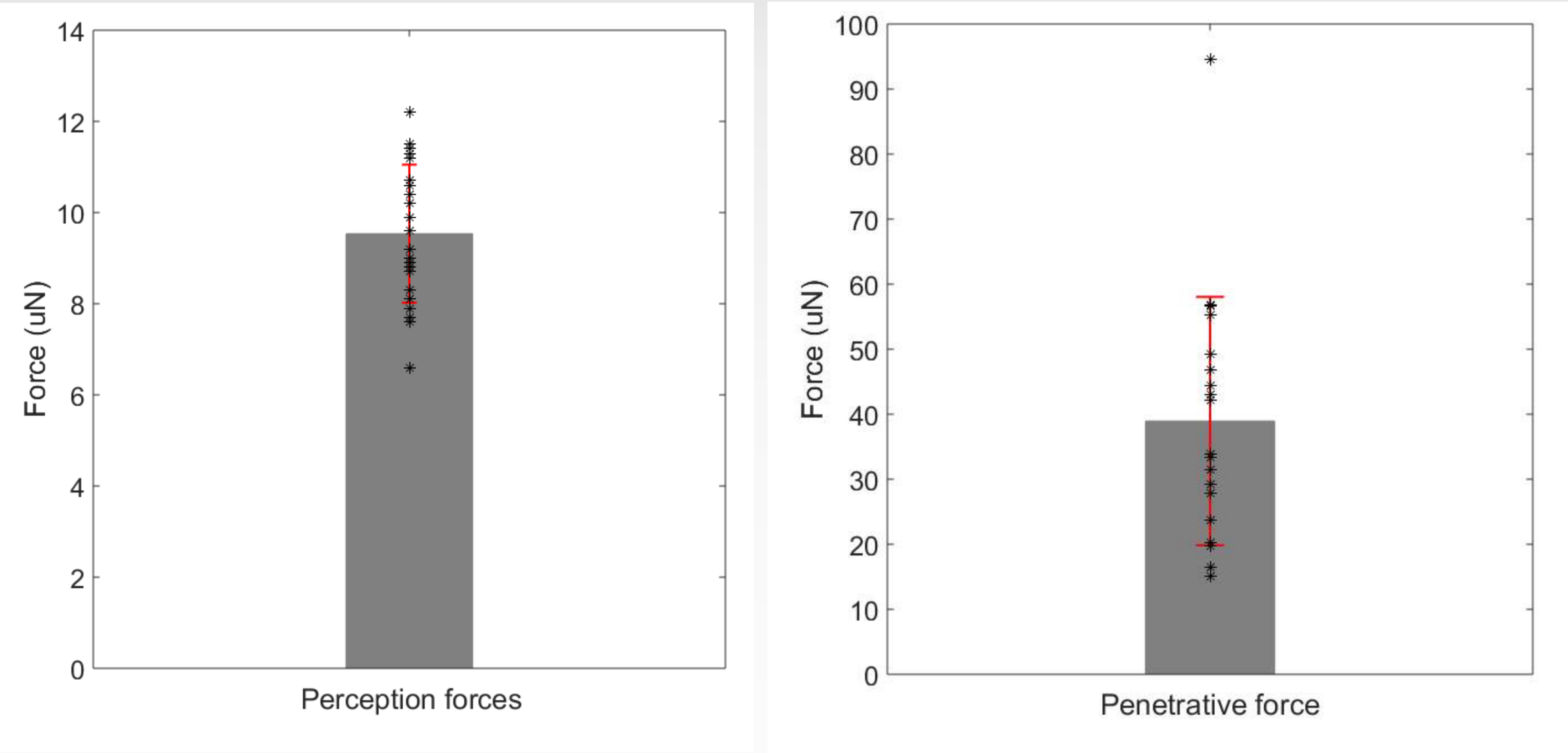
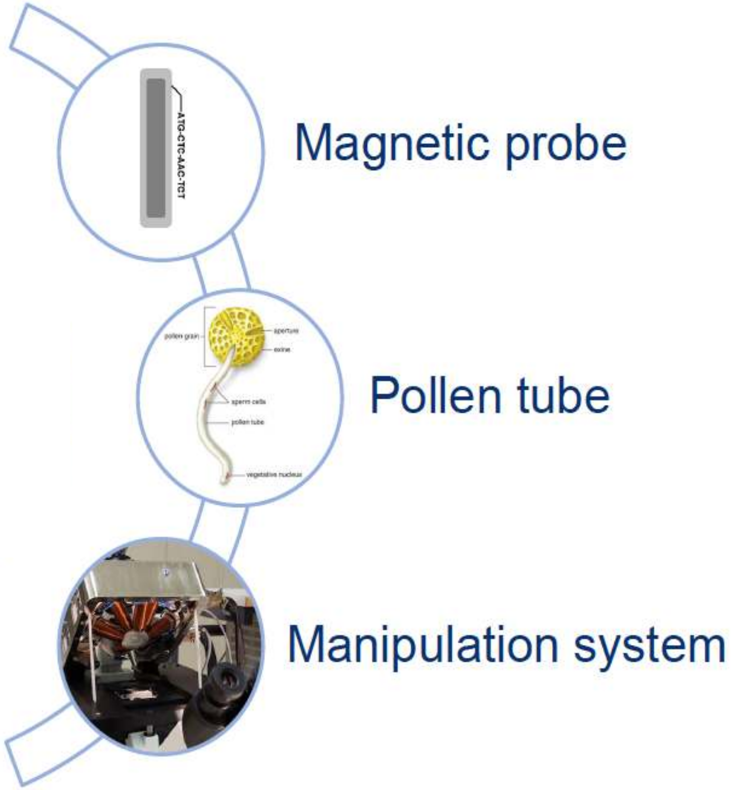
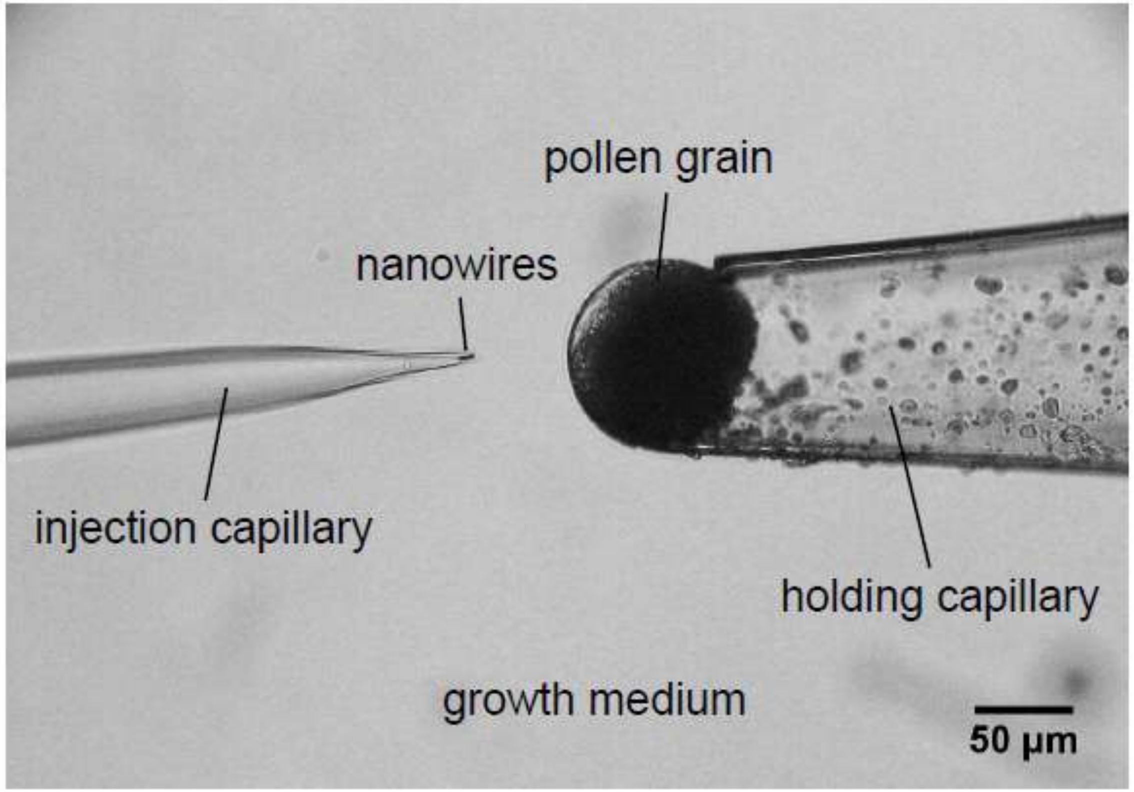
Penetrative Forces up to 60 µN Are Generated by Lily PTs



How does the pollen tube generate this large penetrative force?
Rapid increase of turgor pressure?

Magnetic Nanowires to Measure Internal Biomechanical Properties





Measure and manipulate local, internal properties:

- viscosity of the cytoplasm
- cytoplasmic streaming
- cytoskeleton (local disruption)

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Nanowire Synthesis by Template-Assisted Electrodeposition

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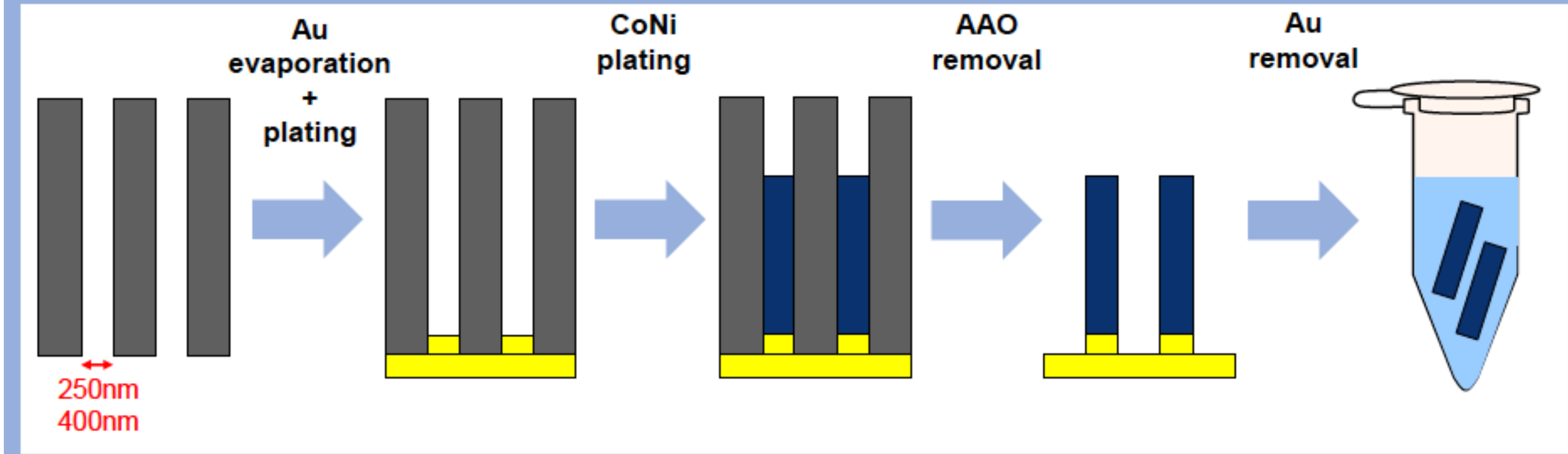
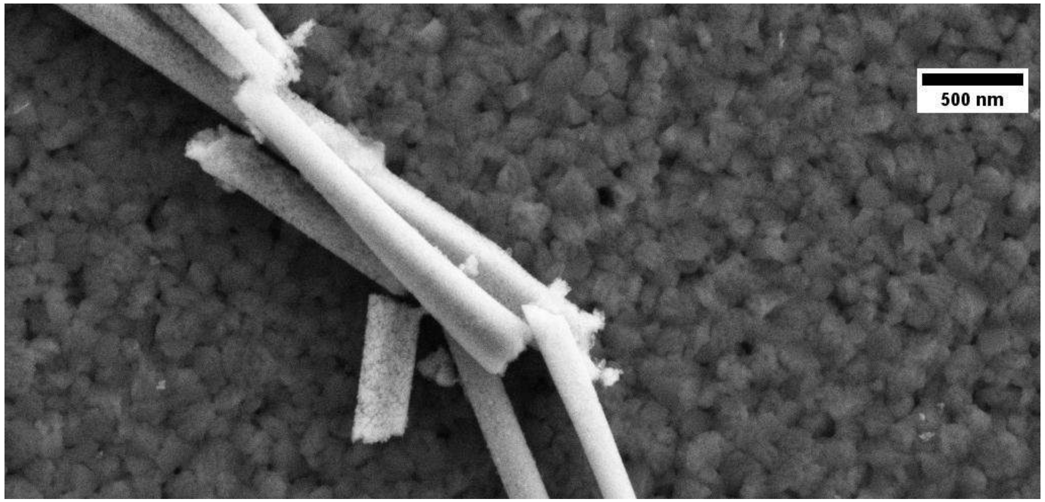
Nanowire synthesis in AAO templates (Anodic Aluminum Oxide)





Sol-Gel Method
To avoid agglomeration and to allow conjugation/functionalization with:

- pH-sensitive dyes (e.g. FITC)
- viscosity-sensitive dyes
- antibodies, proteins, etc.

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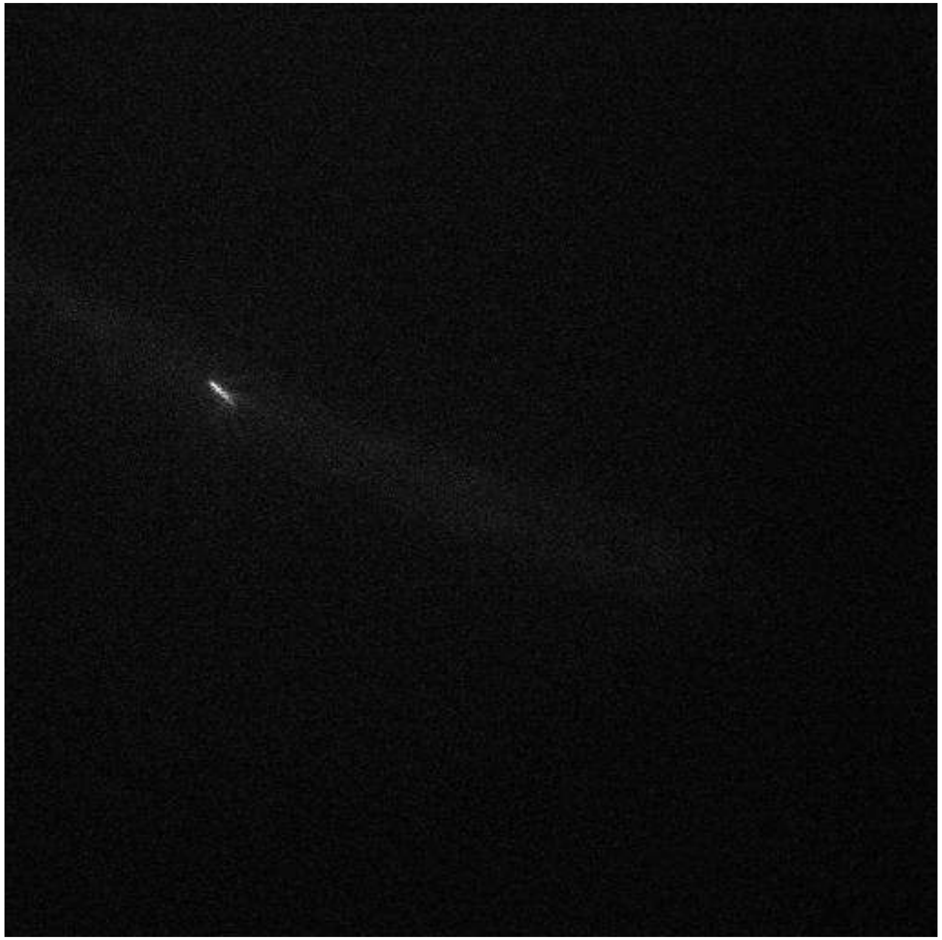
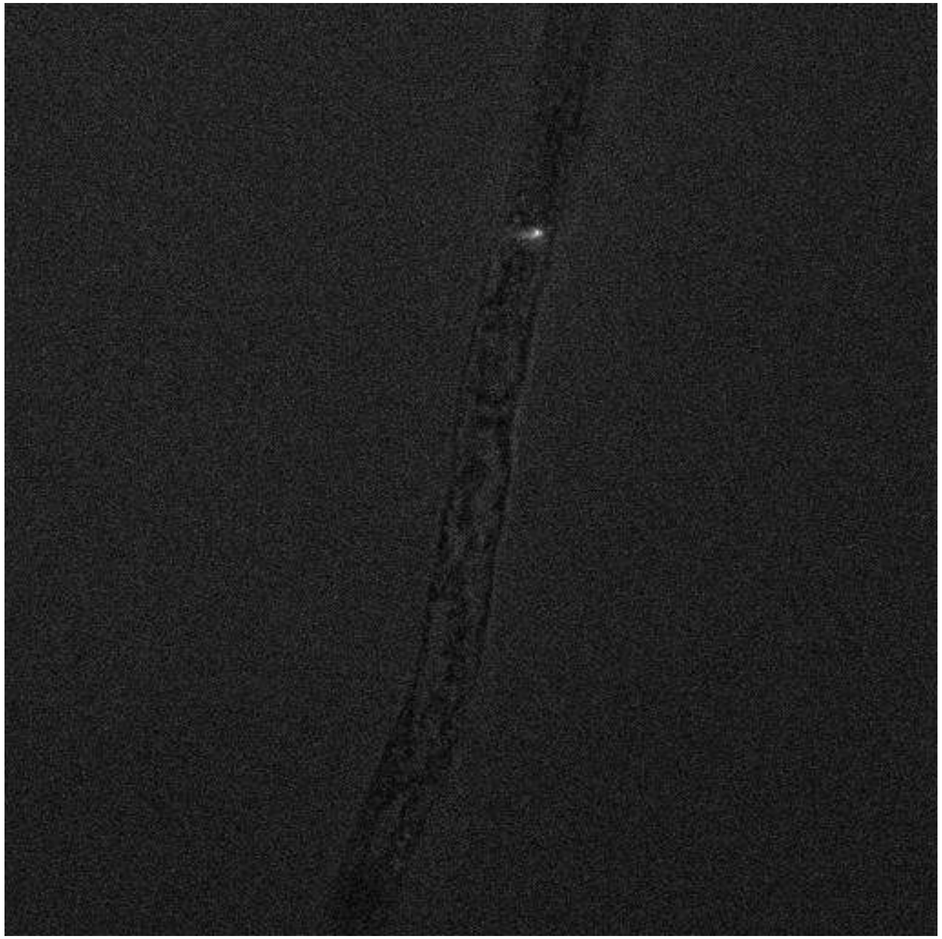
Injection System with Living Pollen Tube Established





Ulrike Nienhaus, Naveen Shamsudhin

Permanent magnet: only rotation possible

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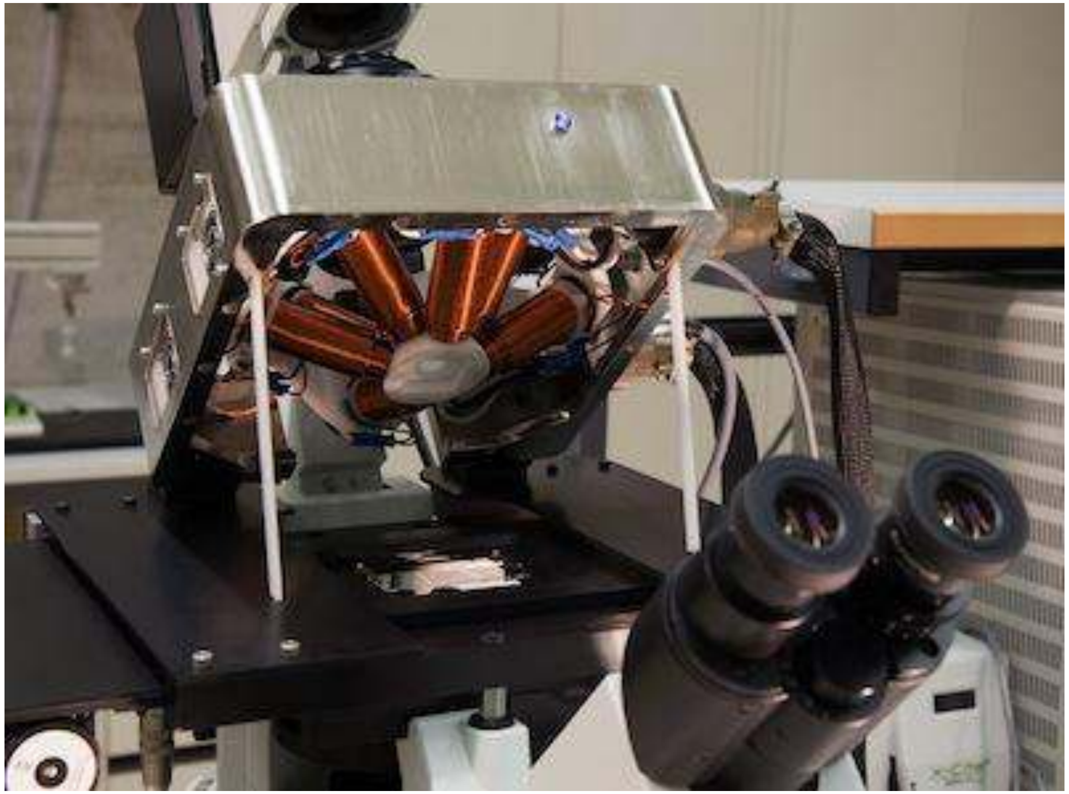
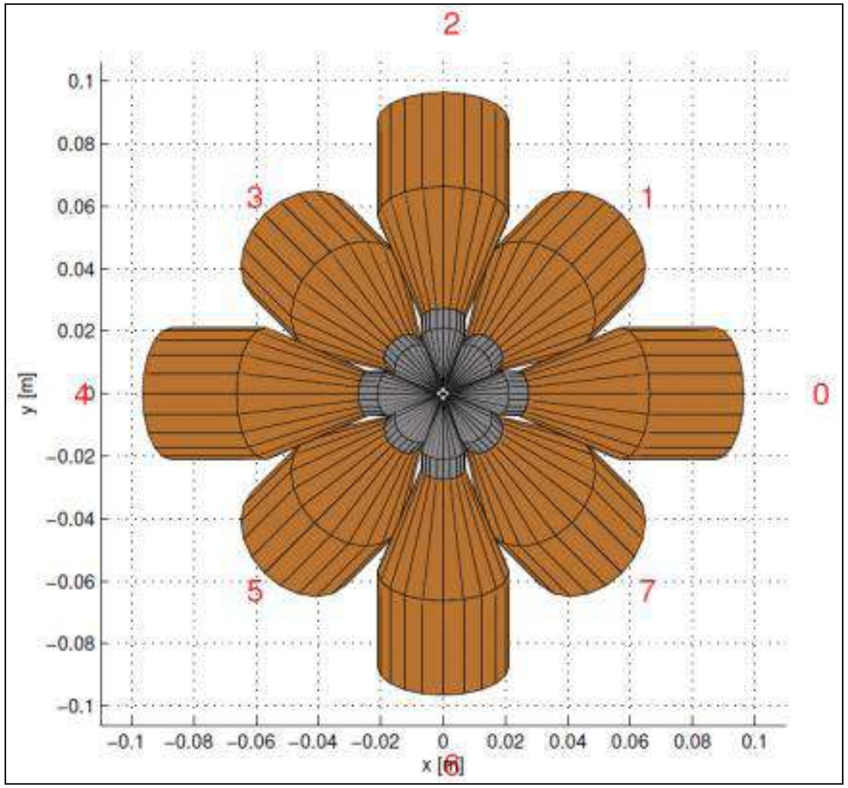
Magnetic Manipulation System: *Nanomag*

MecanX





MFG-100-i (*Nanomag*): F_{mag}= 0.427 pN

- **Gradient fields**
  - to move through the tube
- **Rotational fields**
  - to measure viscosity
- **Core extending tips**
  - to amplify magnetic field 7.5-fold

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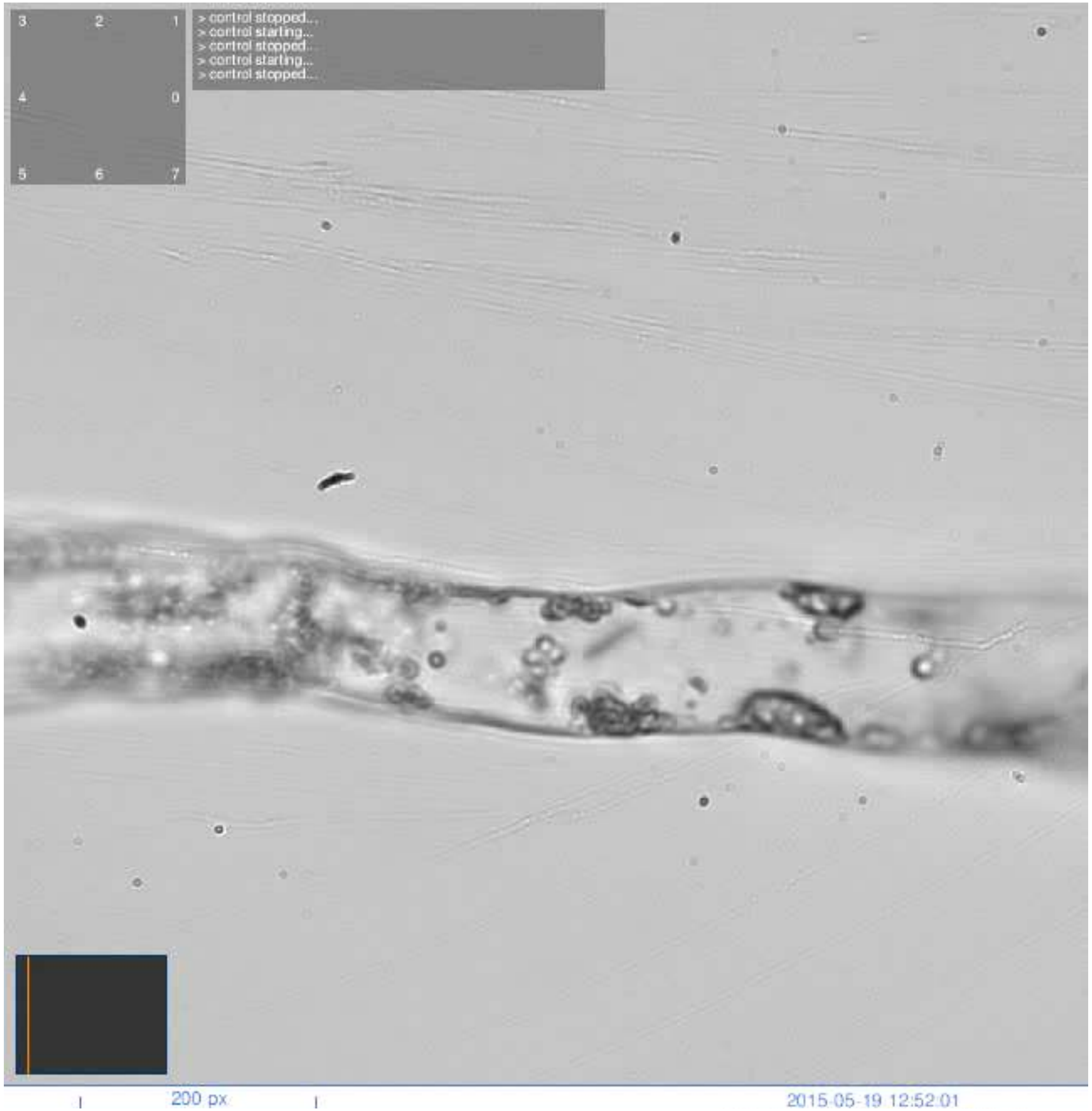
Manipulations with the Nanomag

Ulrike Nienhaus, Naveen Shamsudhin



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Summary

- We have developed new devices to measure mechanical properties at the cellular level (CFM, RT-CFM, soft indentation microfluidics LoC, electrical LoC, magnetic nanowires, etc).
- Using the *Arabidopsis* pollen tube as a model, we could investigate the relationship between biochemical composition of the cell wall and its mechanical properties
- Interfering with cell wall composition also affects turgor pressure, revealing a tight coordination between the cell wall and the physiological processes in the vacuole.
- Pollen tubes are highly regulated, fine-tuned mechanical systems where changes in turgor pressure or mechanical cell wall properties affect growth and can lead to bursting.
- FEM/Monte Carlo-based simulations of cellular properties accurately predict the growth behavior of mutants with altered cell wall composition.

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