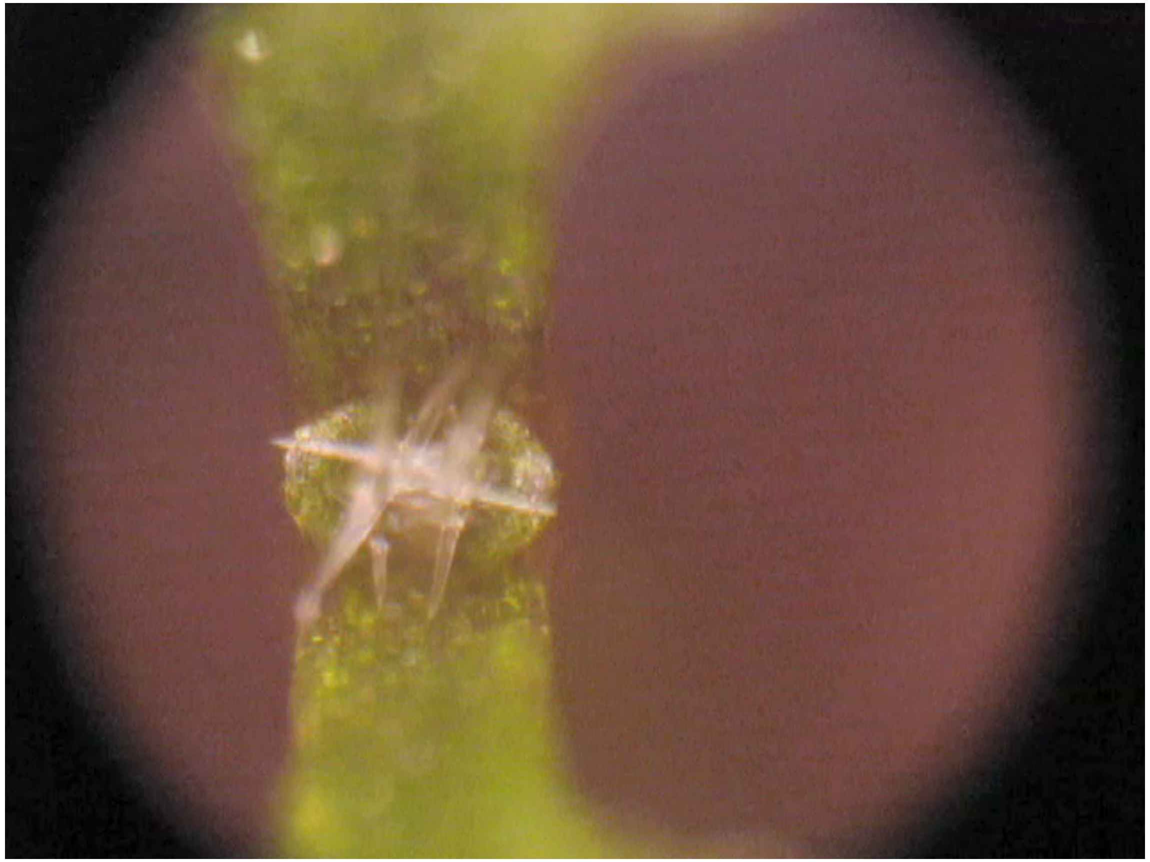
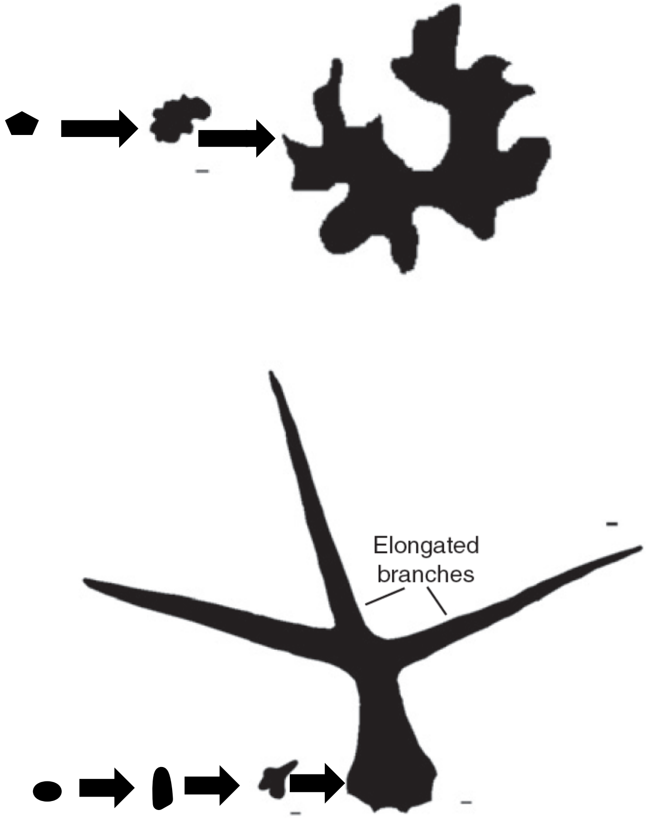
An Integrated Experimental and Computational Approach to Discover the Biomechanical Controls of Leaf Epidermal Morphogenesis



250 µm

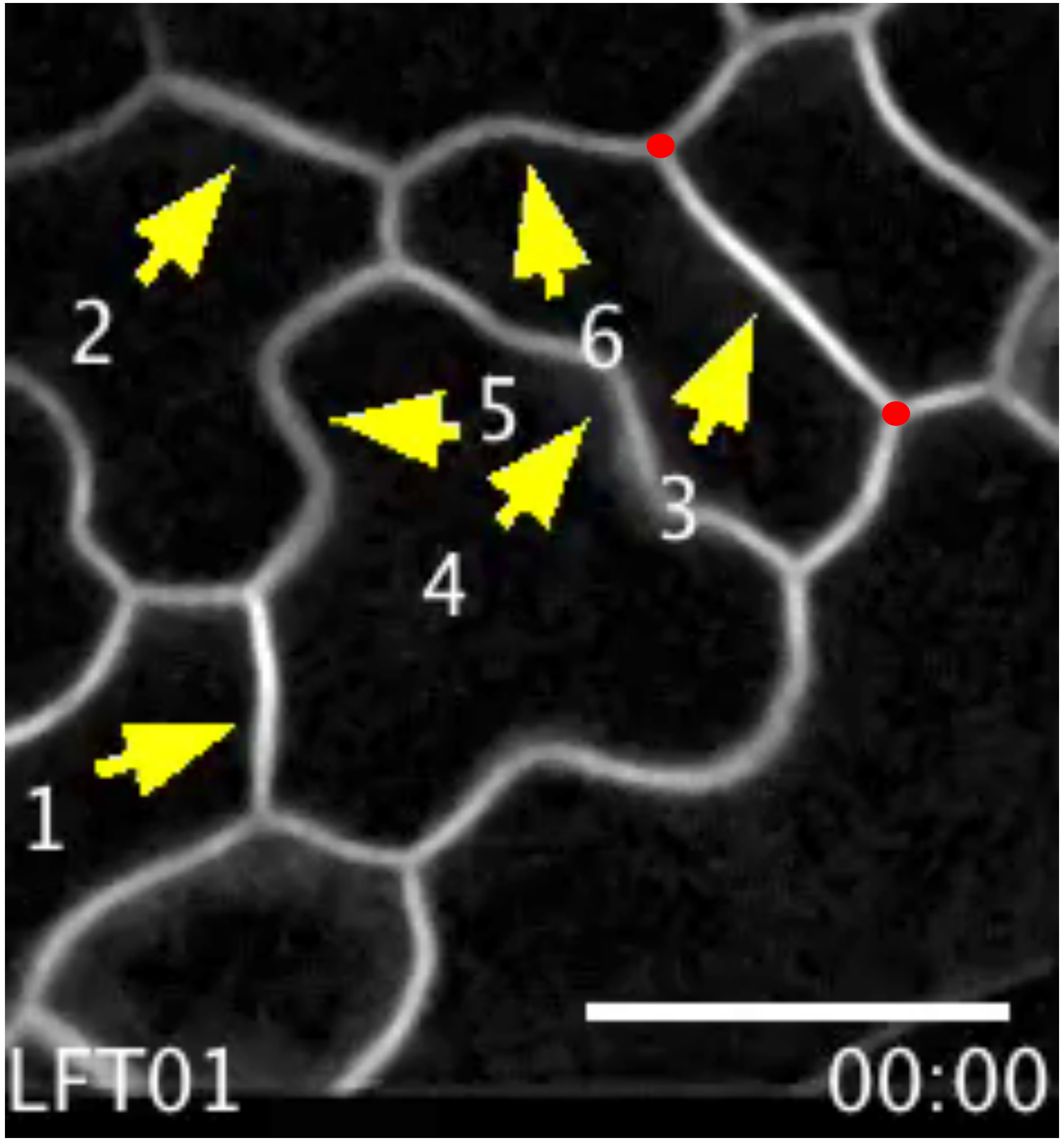
36 hrs

~3000X normal speed



Marks et al. Mol.Plant, 2009

Long-term time lapse imaging of tissue morphogenesis



A top-down modeling approach to drive systems-level analyses of cell and tissue morphogenesis

Parameterization
Validation
Refinement

Experimental Data

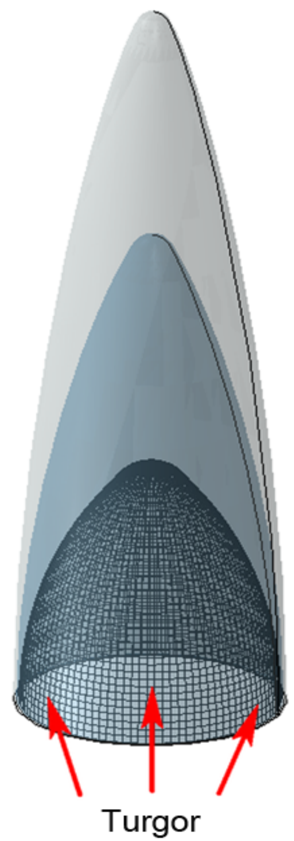
- Multivariate live-cell imaging
- Cell wall mechanical properties
- Cell wall composition
- Mutants
- FP-tagged players

Finite element modeling of the cell Wall

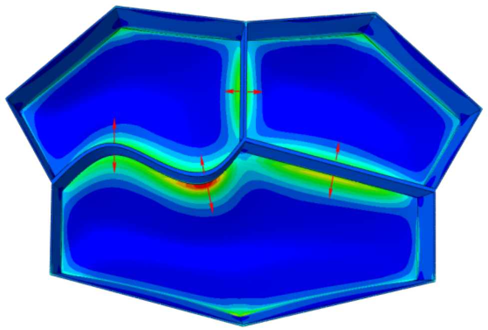
Predictions

Trait engineering

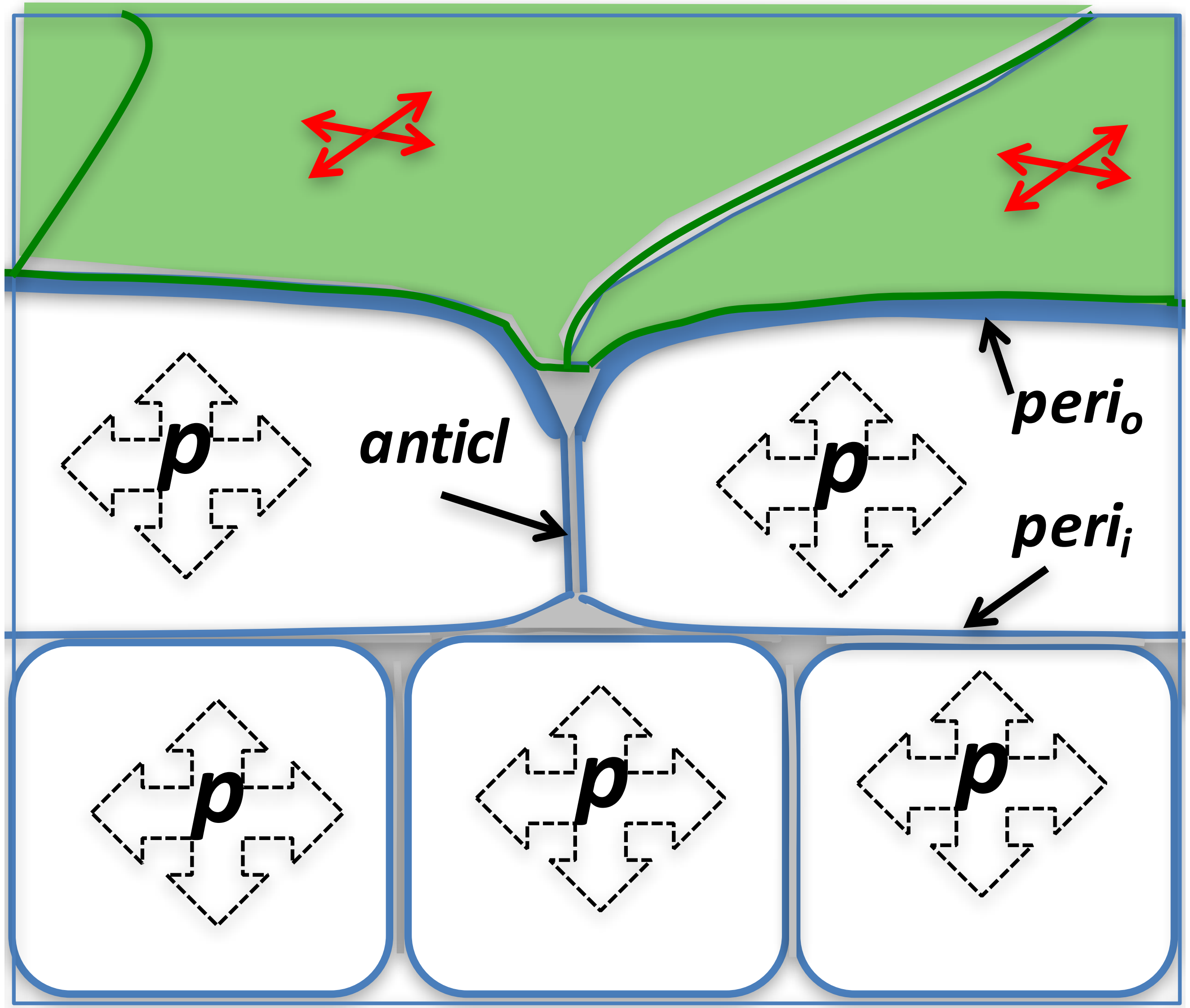
- Cotton fiber diameter
- Leaf size and shape



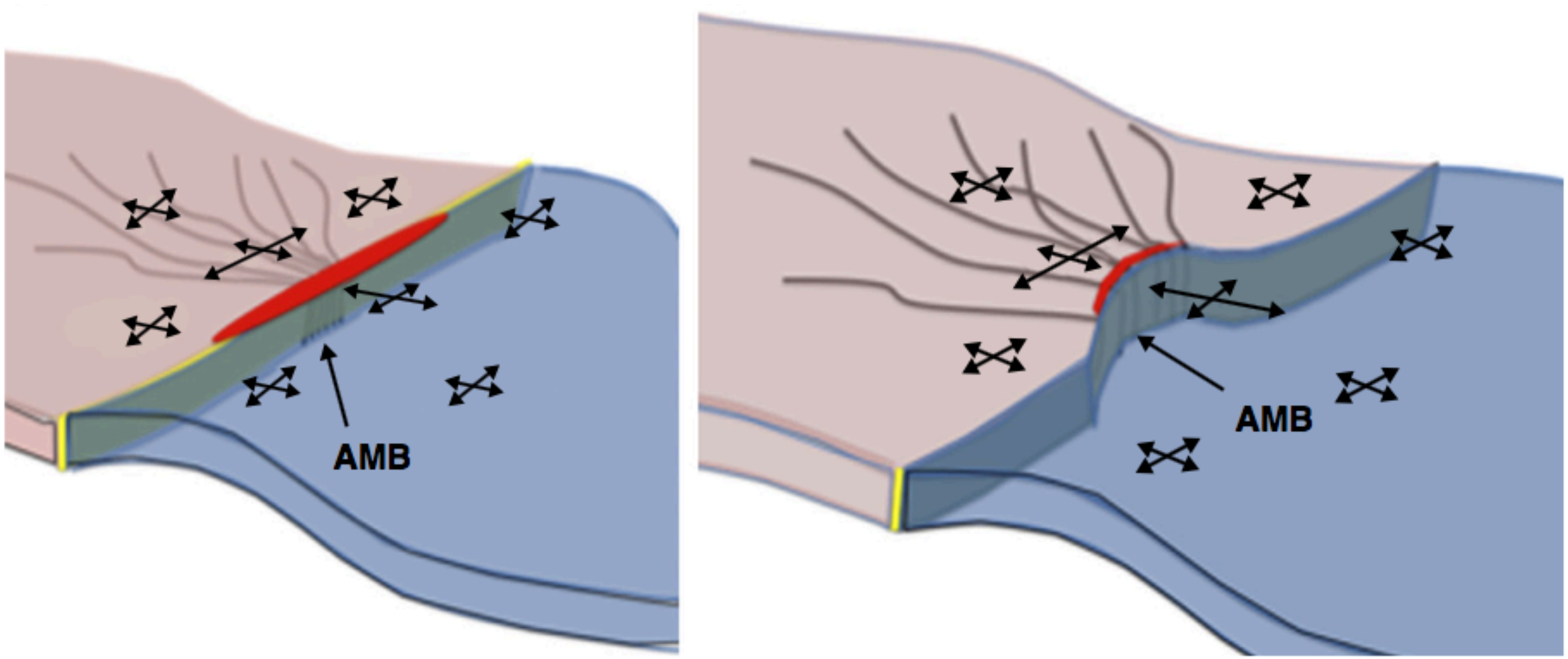
Yanagisawa et al. Nat. Plant, 2015



Wall stress distributions in a turgor-loaded tissue



A biomechanical model for cell lobing and interdigitated growth



MT-cellulose-patterned anisotropic strain

Szymanski, COPB 2014

Pavement cell shape mutants reveal systems-level control of lobe initiation

cellulose synthesis

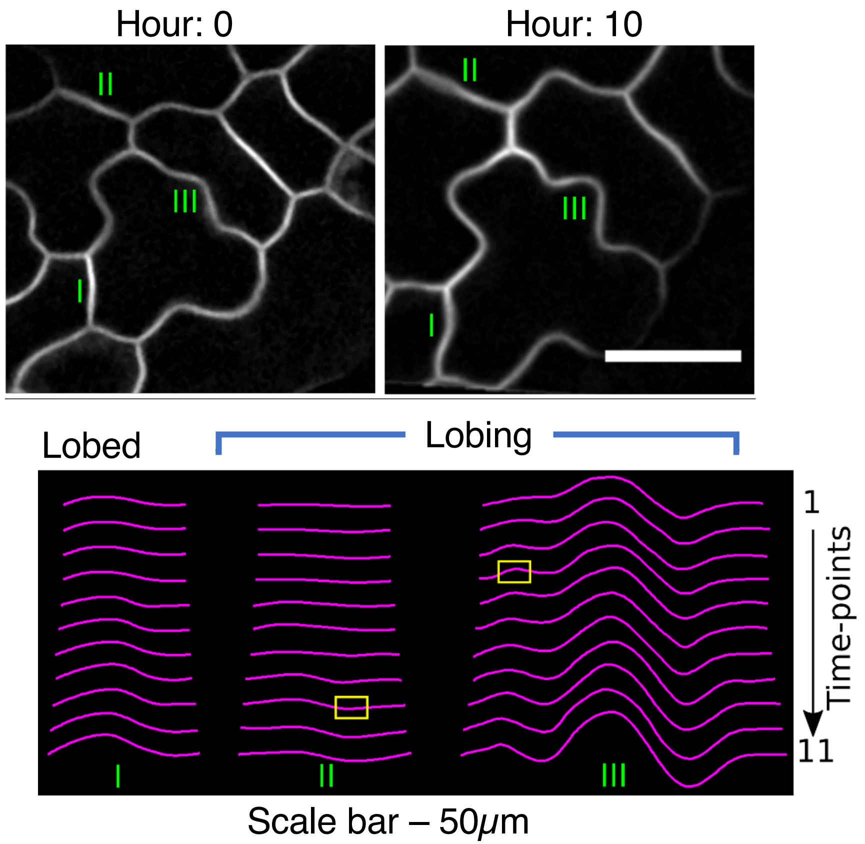
pectin: cell-cell adhesion

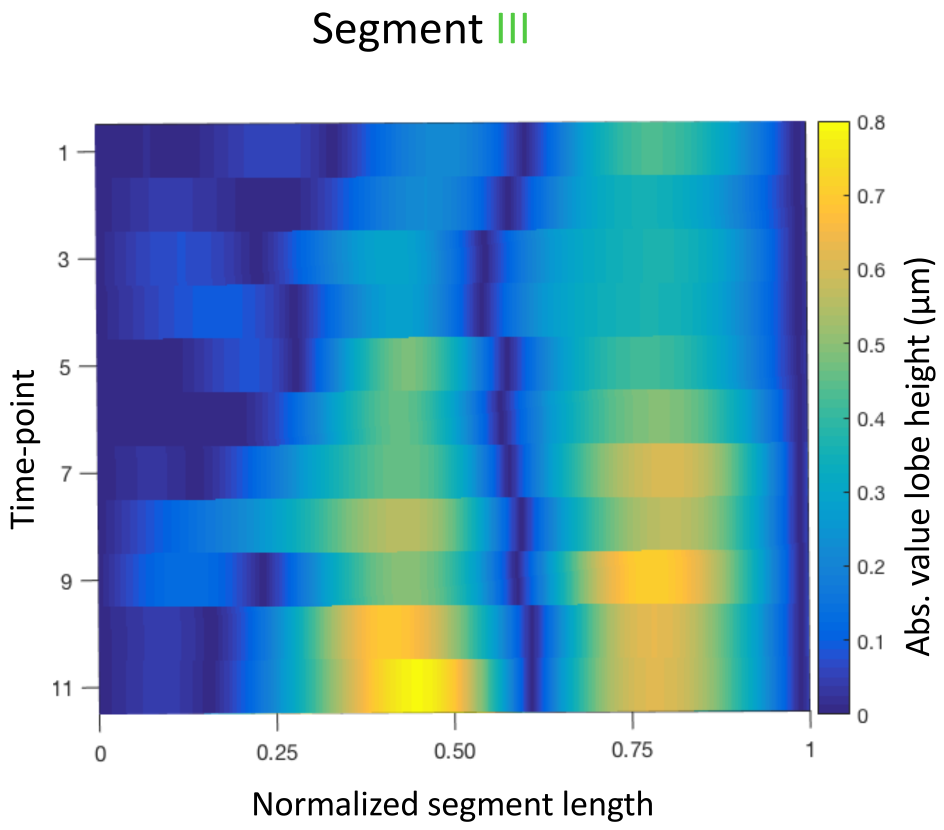
microtubule-associated protein

		Area	Circularity	Lobe Number
Genotype	N	Mean ± S.D	Mean ± S.D	Mean ± S.D
any1	38	10741±3190**	0.59±0.10**	8±2**
clasp1	20	10619±4032**	0.33±0.06**	16±3
exo84b1	23	10746±5138	0.17±0.07**	21±6**
ktn1-2	22	9249±4480	0.34±0.09**	16±2
qua2-1	26	3281±1932**	0.55±0.009**	10±2**
tubg1-1;tubg2-2	25	6377±2289	0.18±0.06**	19±5**
bpp1;2;5	32	4259±1271**	0.72±0.07**	10±2**
WT	30	7863±4215	0.26±0.09**	16±4

*** indicates significant satistical difference betweeen the null mutant and WT by Wilcox-Whitney test (p<0.05)*

A coordinate system to graph cell segment morphogenesis



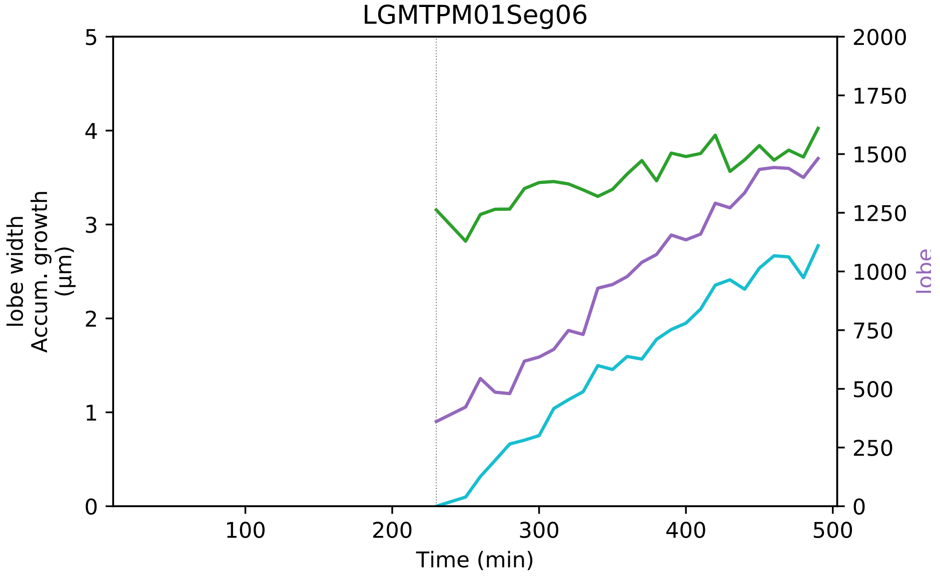


Belteton et al. Plant Physiology, 2018

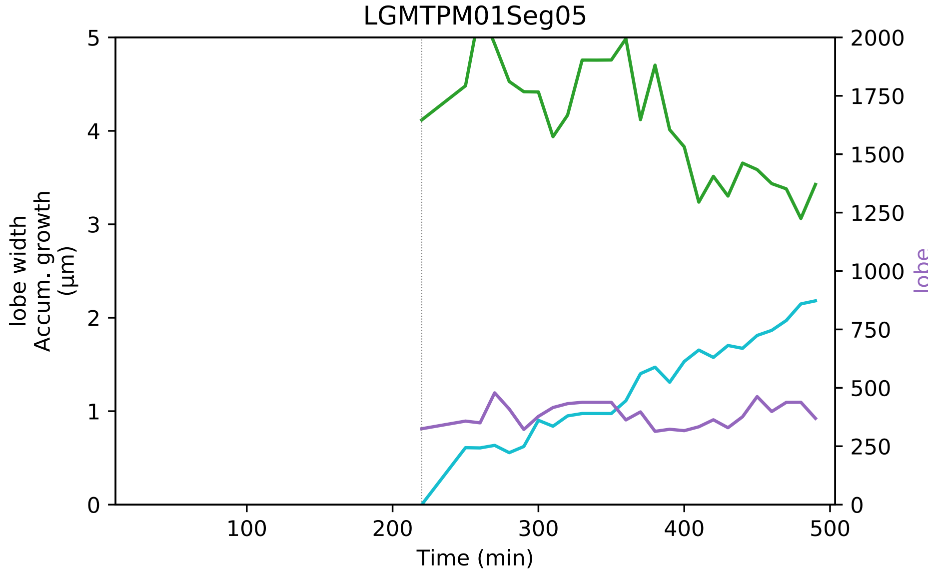
Time-lapsed analysis of cell segment and lobe morphogenesis

green: lobe width
magenta: lobe height
aqua: : segment growth increment following initiation

active lobe features (~30 % 5/17)



static lobe features (~70% 12/17)

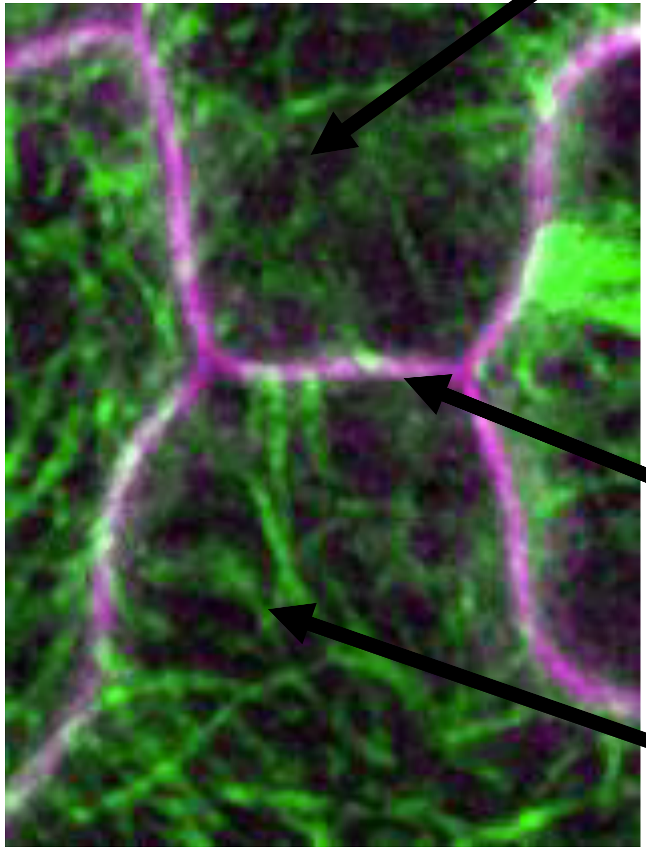


Spatiotemporal analysis of trans-facial microtubules

Plasma-membrane: magenta
Microtubules: green

xy view: projected images

leading cell



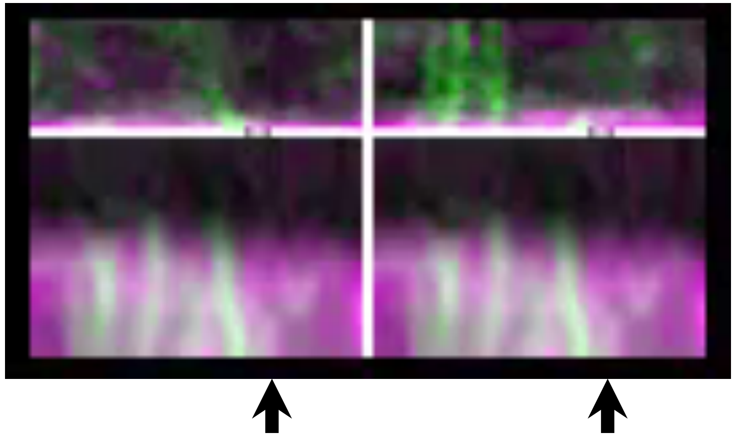
lobe initiation site

responding cell

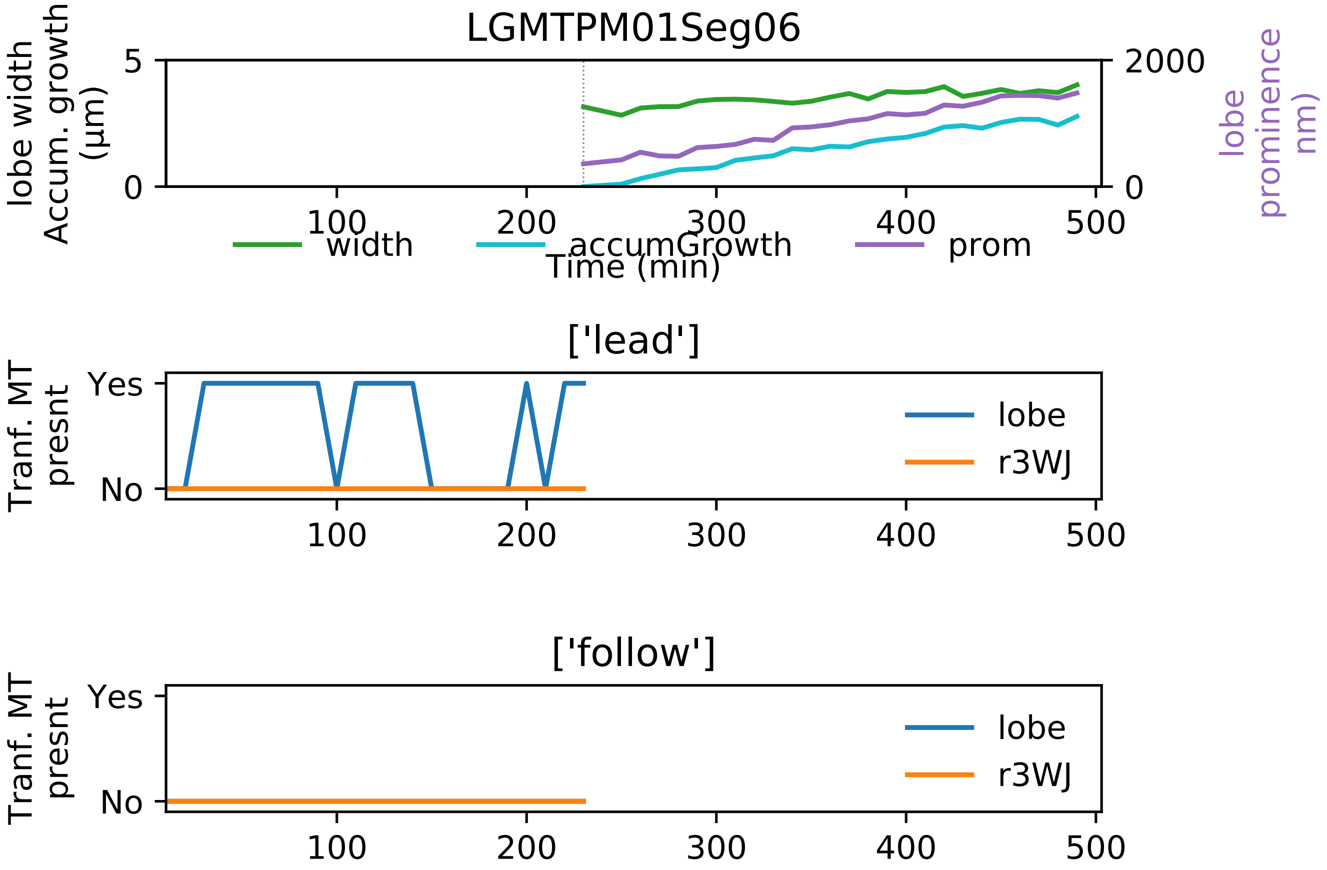
leading cell   responding cell

xy-periclinal

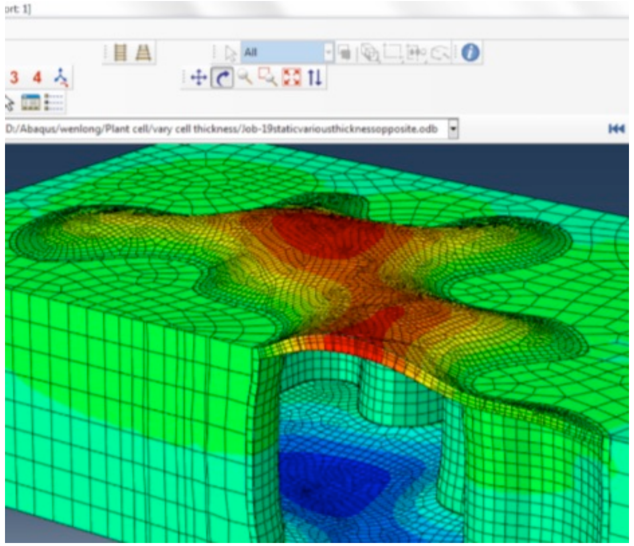
xz-anticlinal

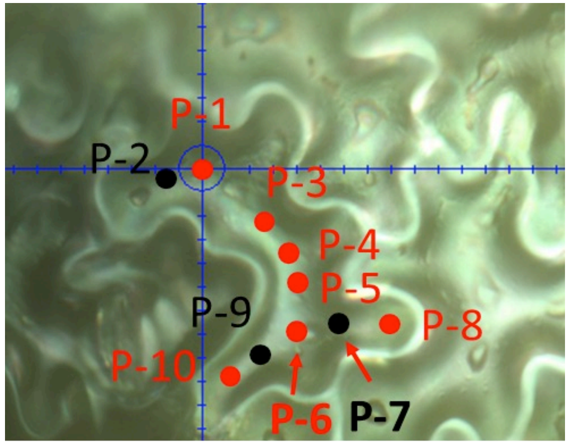


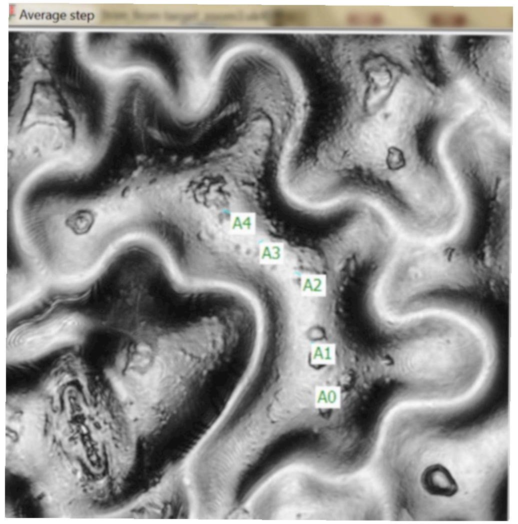
Cross correlation of trans-facial microtubules and lobe formation



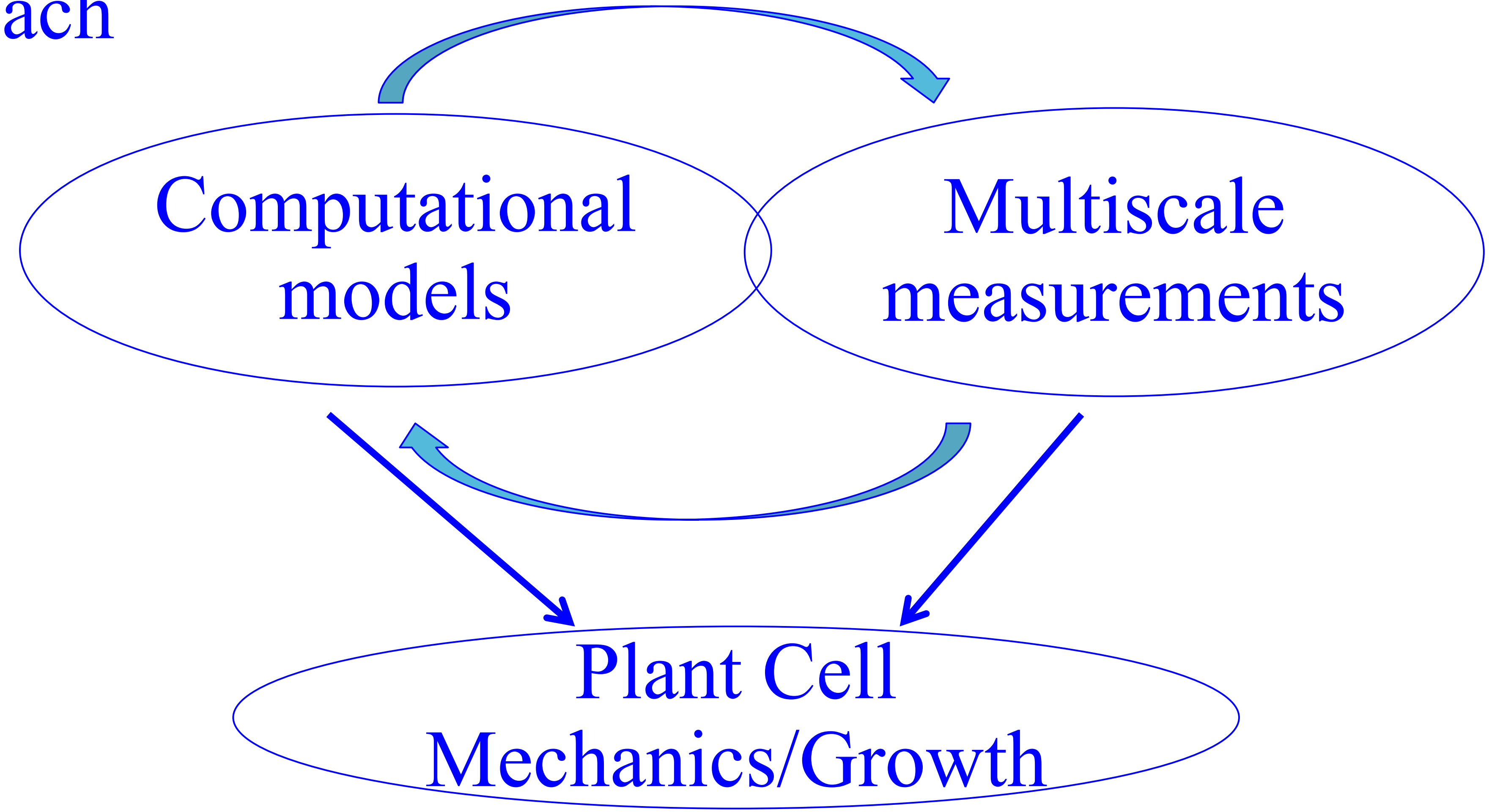
Computational modeling and experimental verification







Approach



Our approach: apply engineering methods to living plants to create <u>predictive</u> computational models

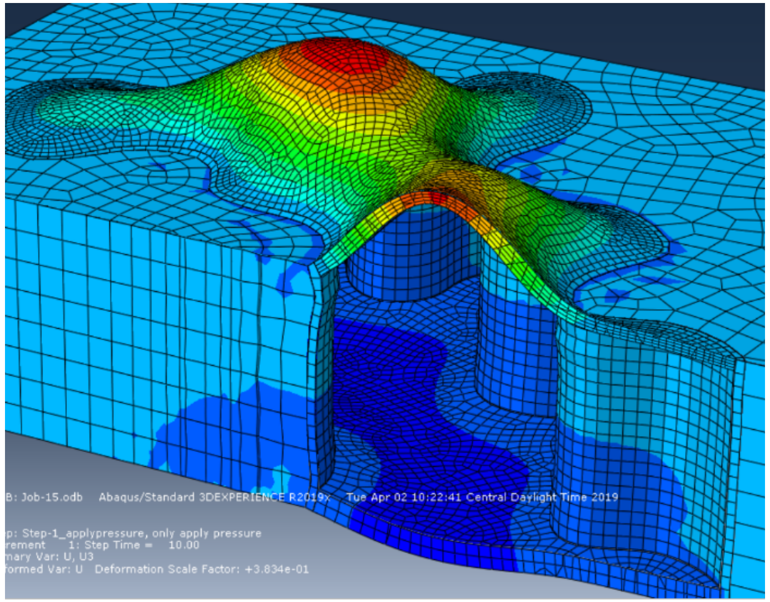
Viscoelastic Properties (time dependent)

- Viscoelastic materials have properties that are dependent on the loading rate and temperature
- Most biological materials have a time dependent mechanical response for a given biological state
- Many examples of viscoelasticity for small and large displacements

Important for both computational modeling and measurements

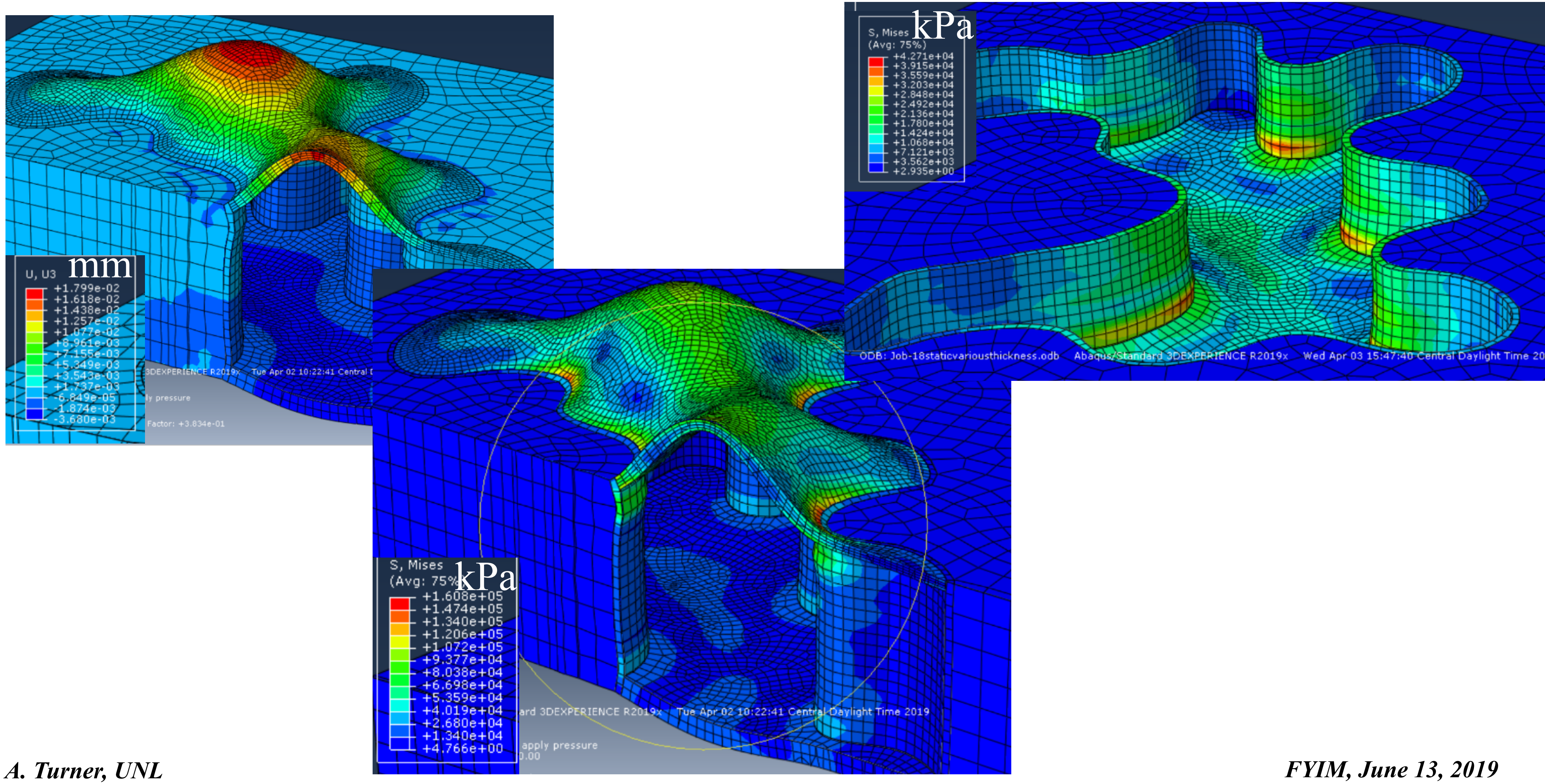
Computational Modeling

- Finite element method (FEM) is an approach used to solve partial differential equations that are used to model a physical system
- Geometry is discretized spatially and a model for the local material response is needed
- Outputs include displacement, stress, and strain
- Parameters per element (global heterogeneity)
  - Material symmetry (isotropic or anisotropic?)
  - Viscoelastic properties
  - Linear/nonlinear; small/large strains

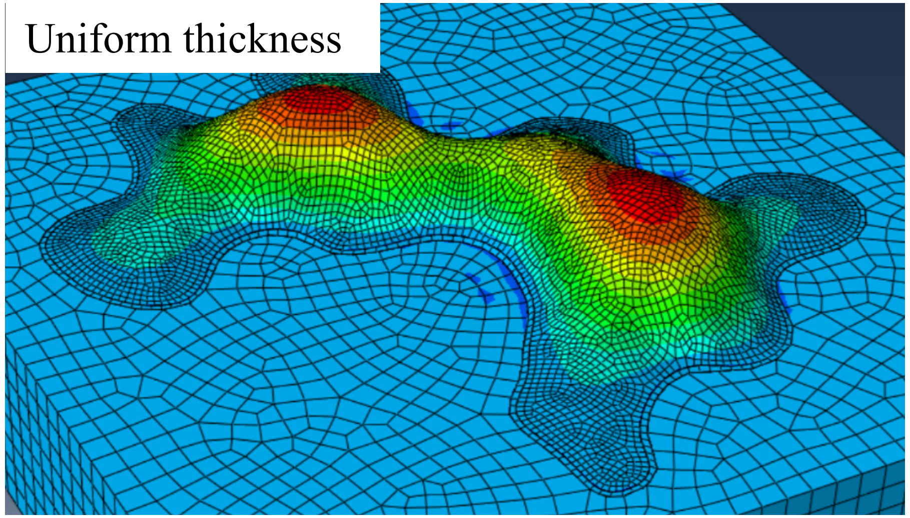


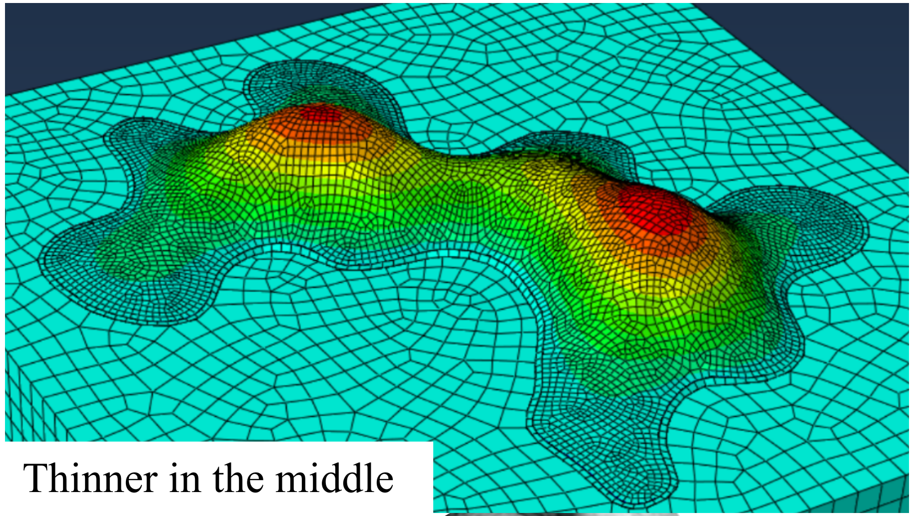
Start with simple model and add complexity as needed;
use measurements when possible; sensitivity studies otherwise

Example result for a pavement cell

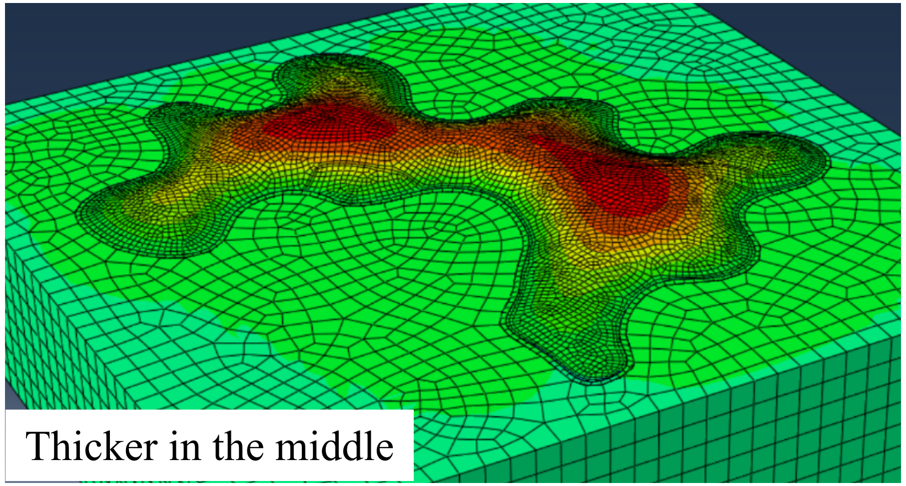


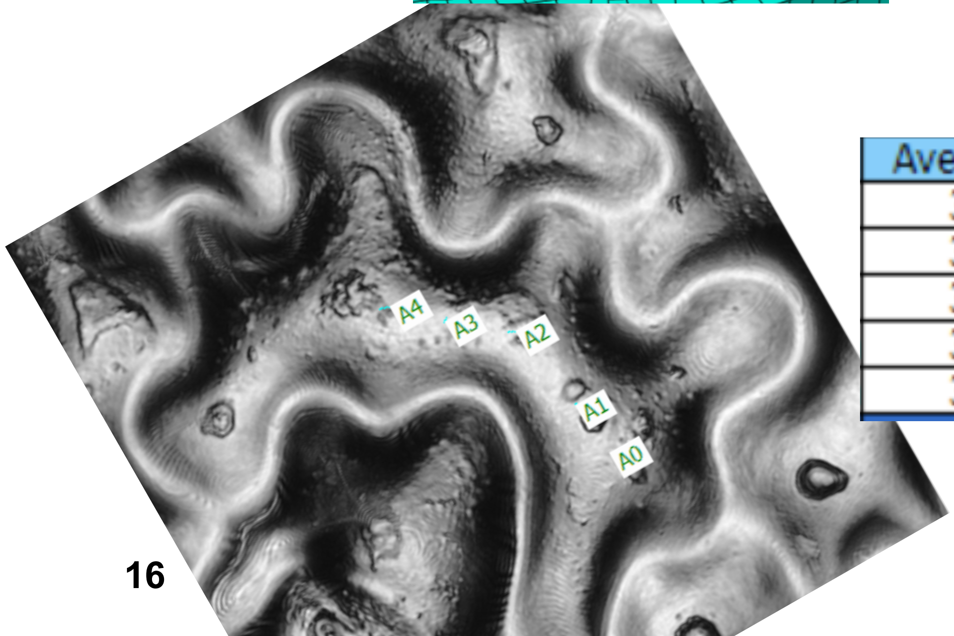
Height variation of the periclinal wall


Uniform thickness


Thinner in the middle

Turgor pressure:
0.8 MPa
Thickness:
0.7~1.2 μm


Thicker in the middle



Ave. height
34240nm
34800nm
34929nm
34279nm
33630nm

How to determine the mechanical properties to use for the model?

Characterizing Mechanical Properties at the Micro- and Nanoscales

Mechanical Properties using Touch

- The nanoindenter and atomic force microscope are examples of instruments that use a tip in contact with a sample surface for imaging and for determining mechanical properties (scanning probe microscope, SPM)
- Many examples from every day life
- Quantitative vs. qualitative

Quantitative measurements require robust models of the entire measurement system

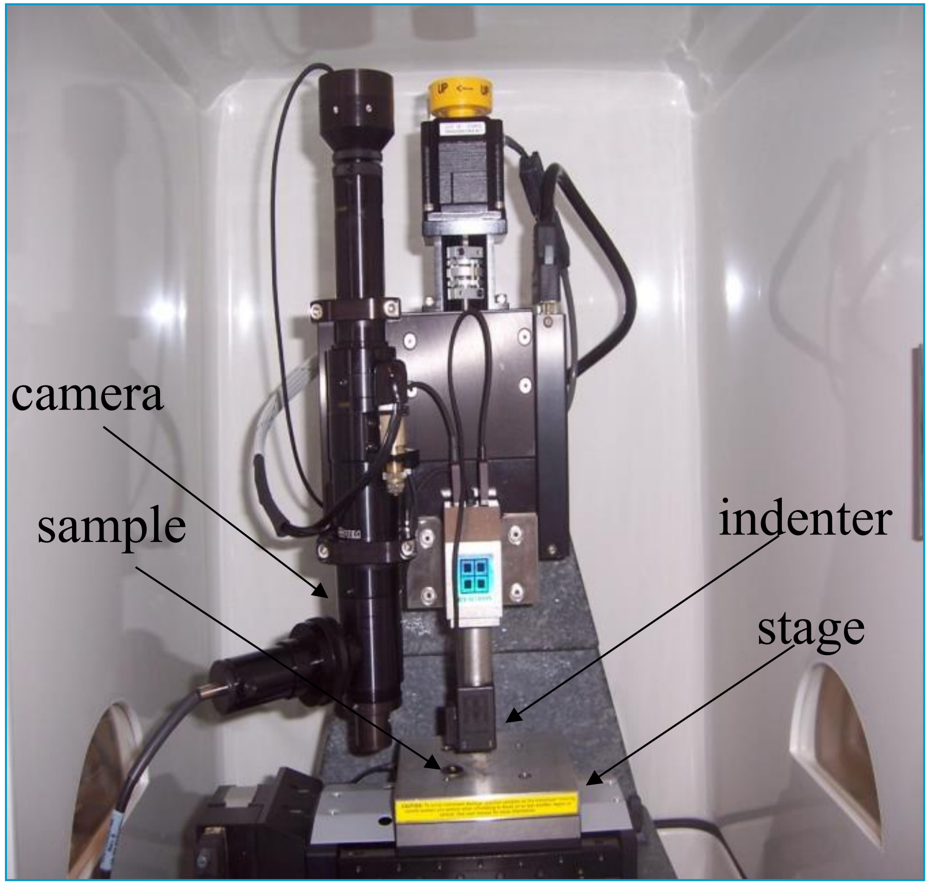
Length Scales

- Production of new polymers – characterization of the properties of these materials is important for their performance
- Polymer blends have many interfaces and overall performance is often controlled by interfacial behavior
- Polymer behavior at small scales may uncover new details about deformation mechanisms, polymer organization, polymer breakdown, etc.

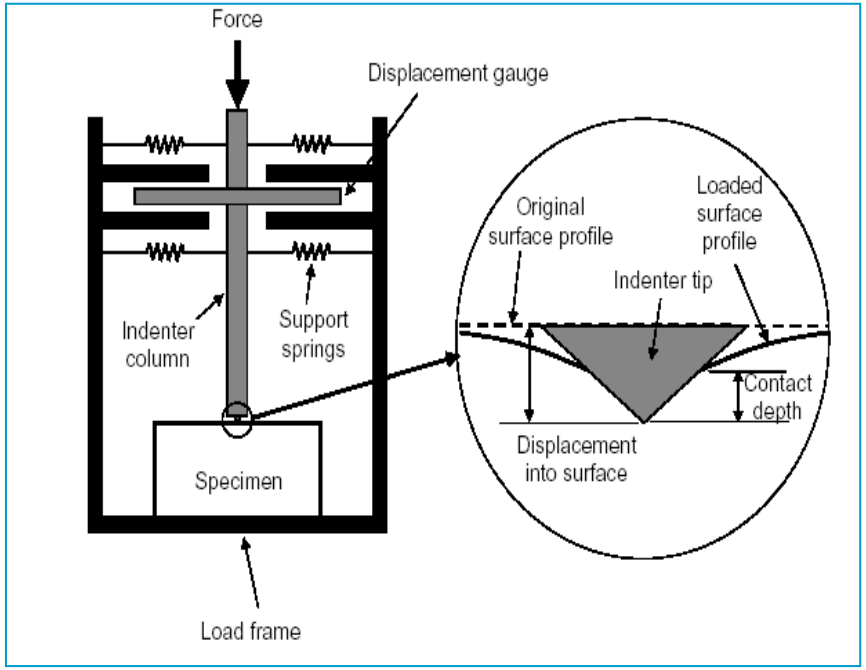
Scales: approximate number of macromolecules involved in test

Macroscale	Microscale	Nanoscale
(DMA)	(nanoindenter)	(atomic force microscope - AFM)
10^{16}	10^{6}	10^{2}

Nanoindentation



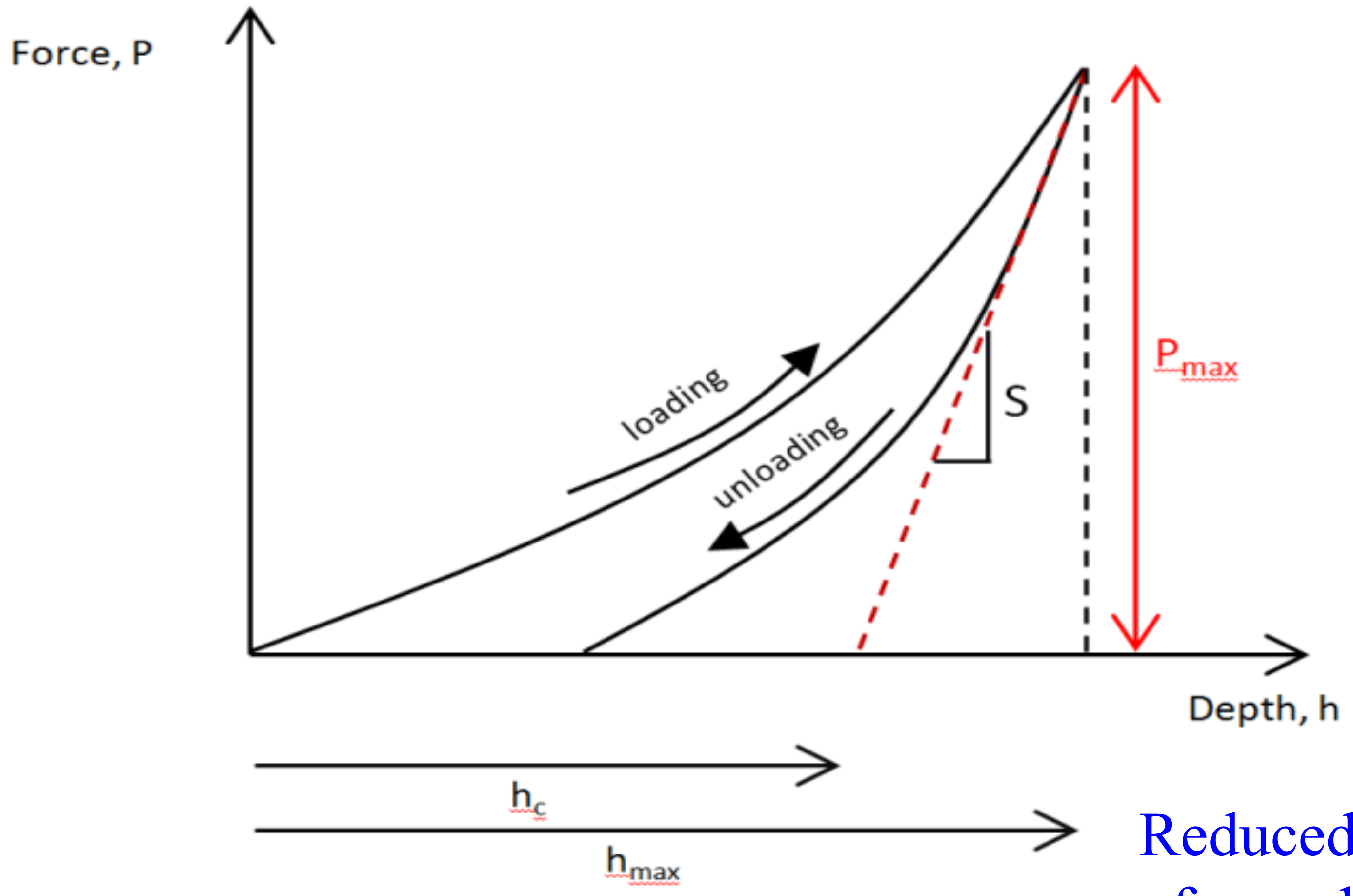
Hysitron Triboindenter 950



Schematic diagram of the nanoindentation system (Odegard et al.)

Precision control of force (nano-Newton resolution) or displacement (nanometer resolution); custom tips

Quasi-Static Measurement



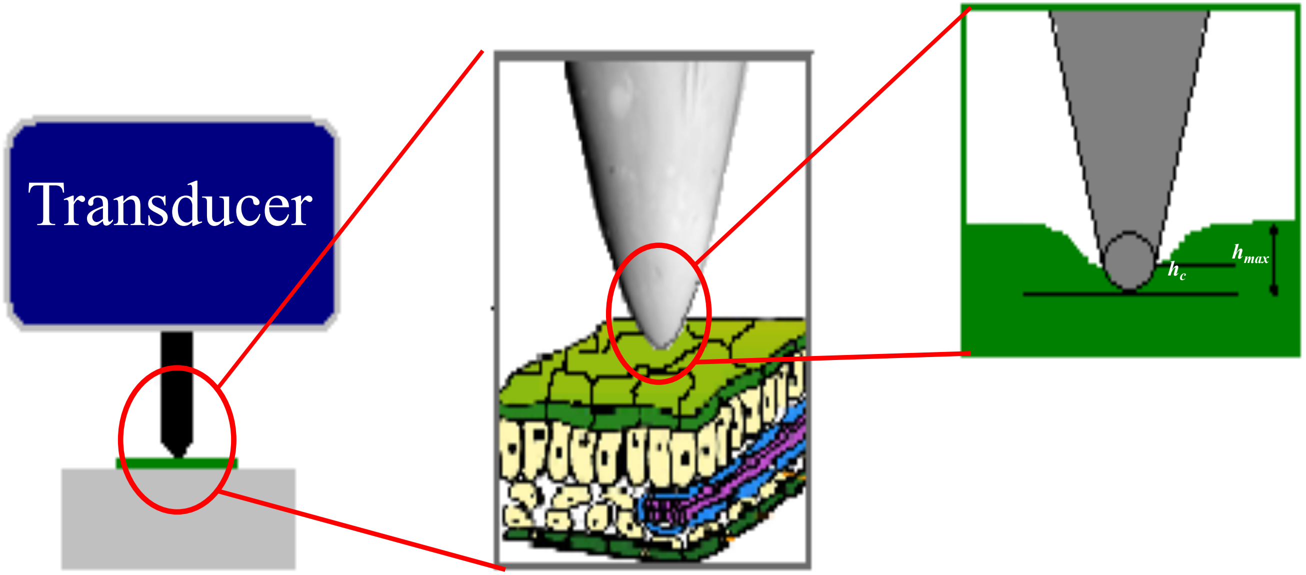
$$H = \frac{P_{max}}{A}$$

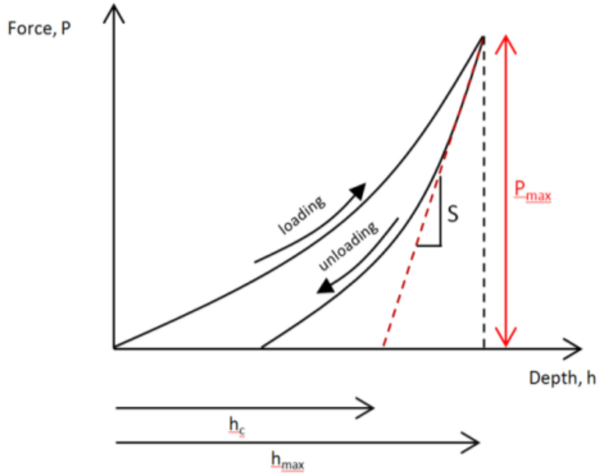
$$Er = \frac{S}{2}\frac{\sqrt{\pi}}{\sqrt{A}}$$

Contact area needed

Reduced modulus is a combination of sample and tip properties

Nanoindentation on Plant Cells





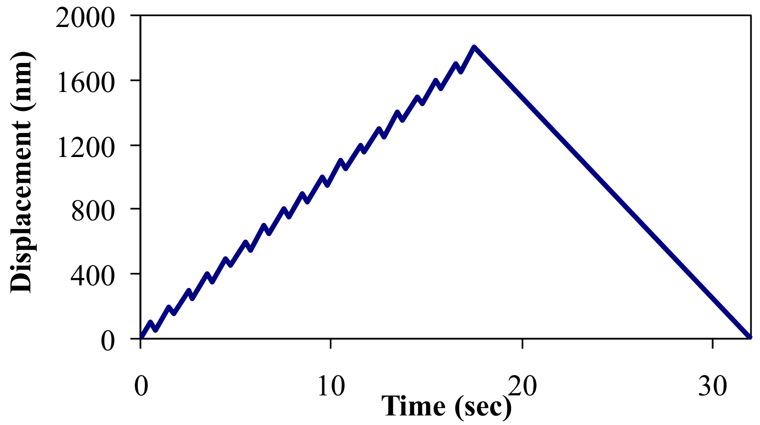
More complex analysis of deformation:

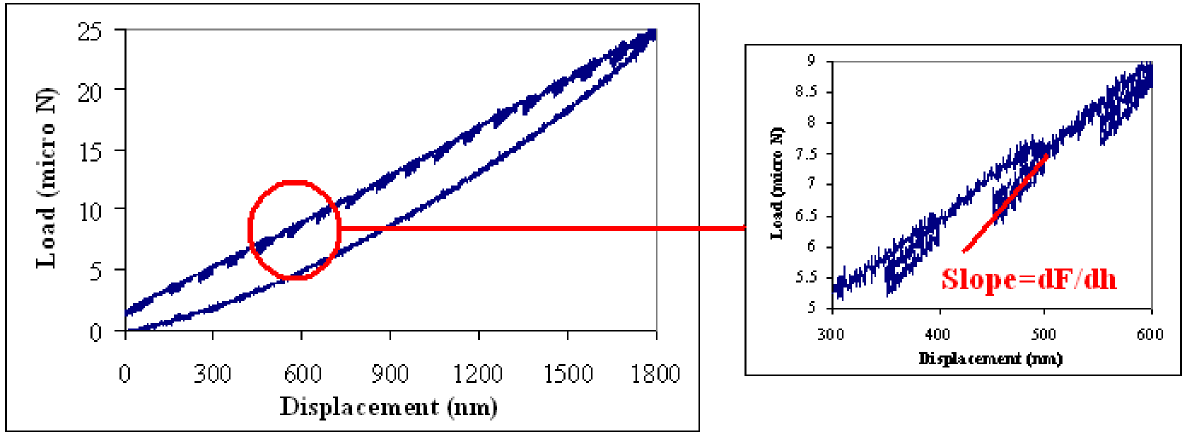
Thin wall

Internal turgor pressure

Wall bending + wall compression

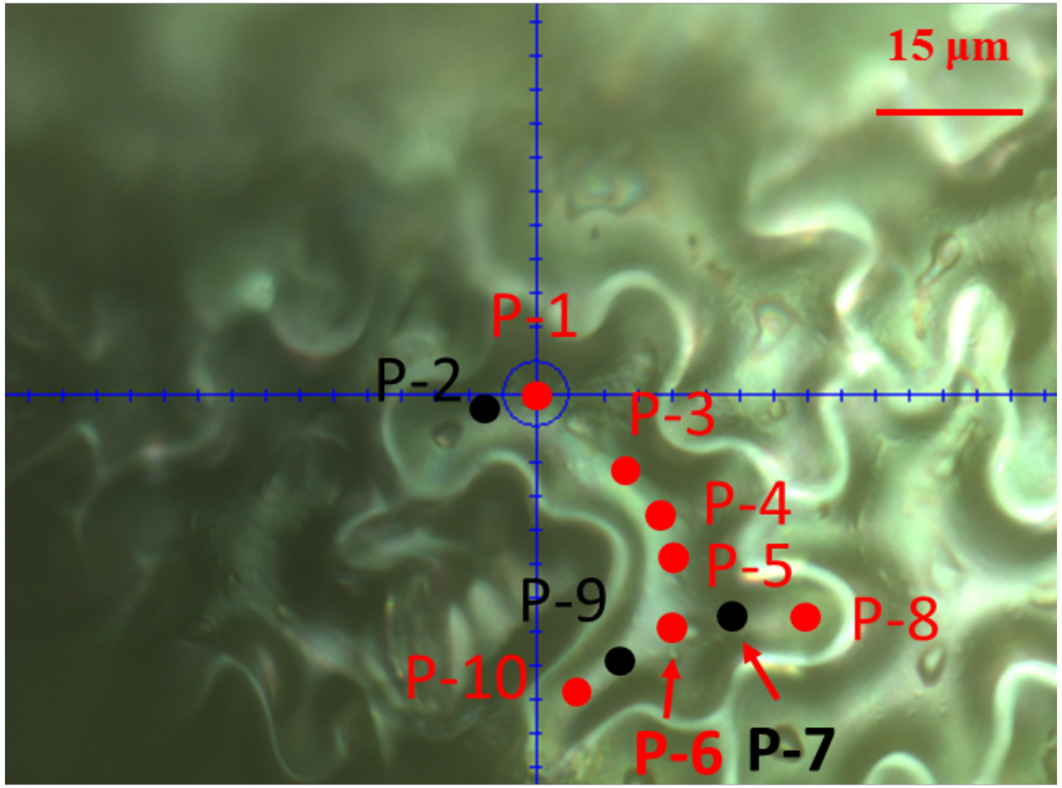
Cyclic, depth changing measurements

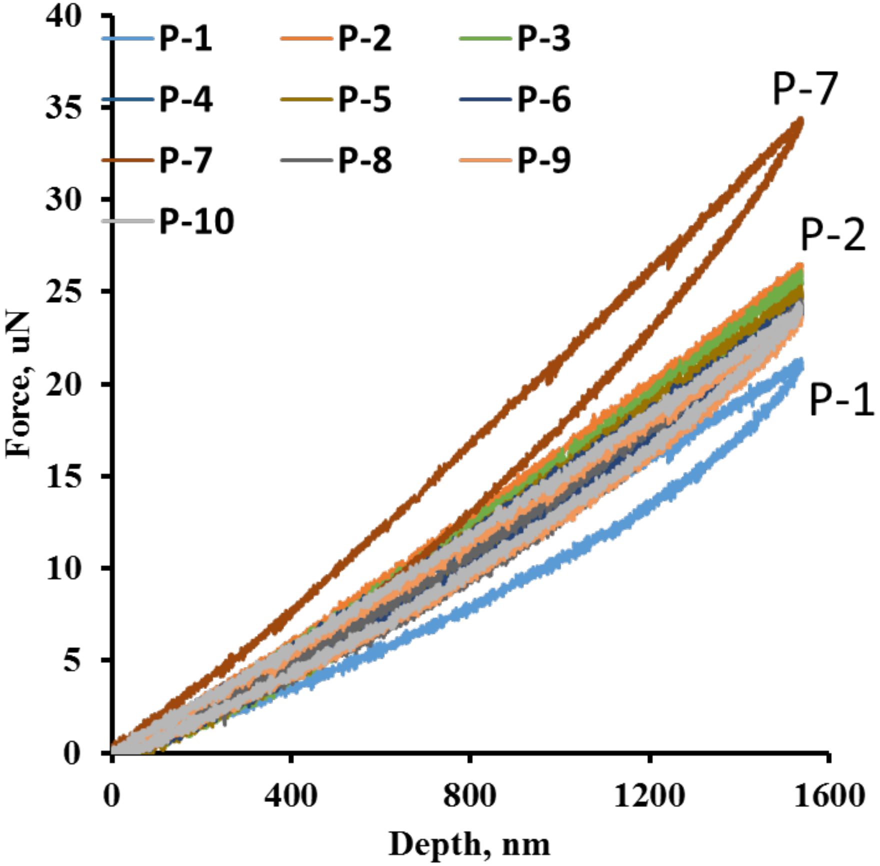




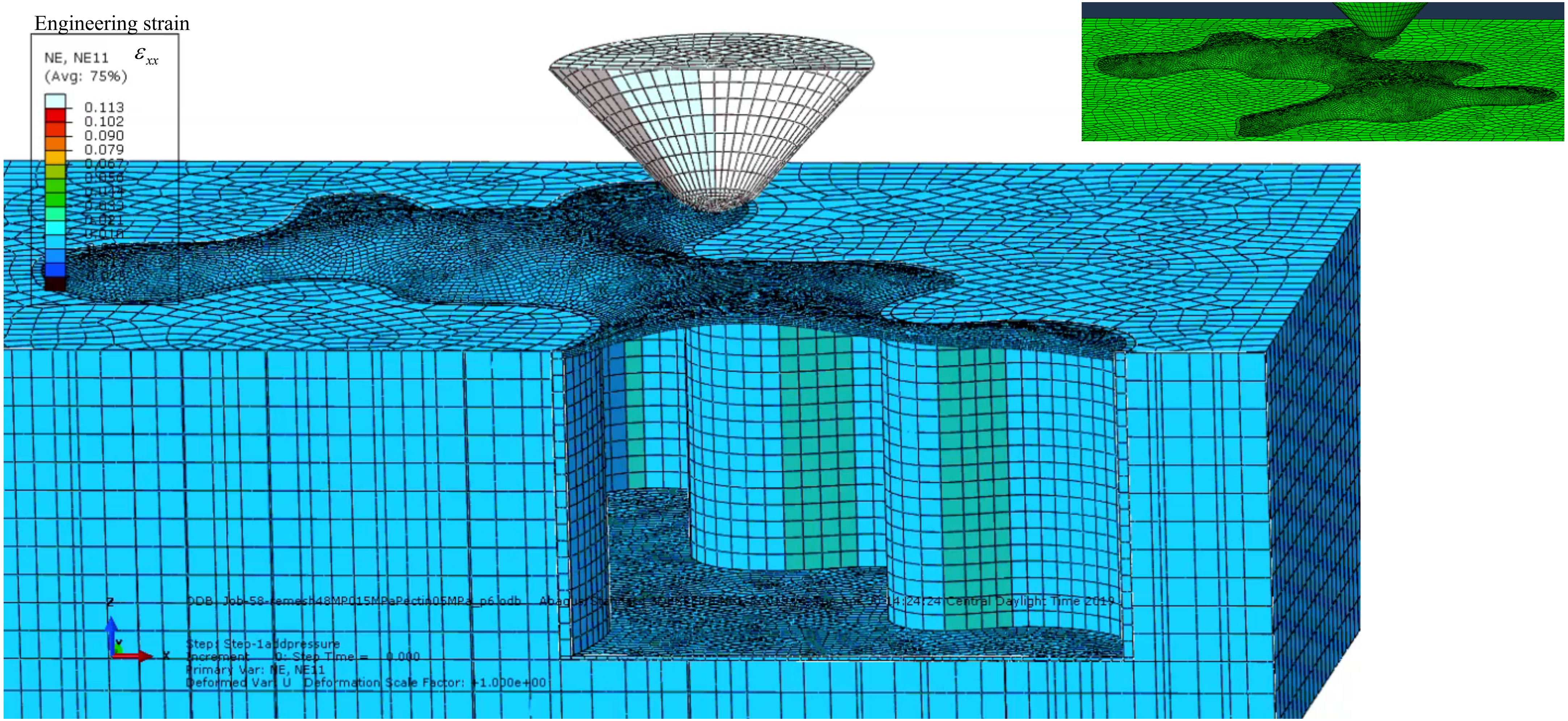
E. Forouzesh, A. Goel, S. A. Mackenzie, and J. A. Turner, "In vivo extraction of Arabidopsis cell turgor pressure using nanoindentation in conjunction with finite element modeling," *The Plant Journal* 73, 509-520 (2013).

Nanoindentation at different locations of a pavement cell



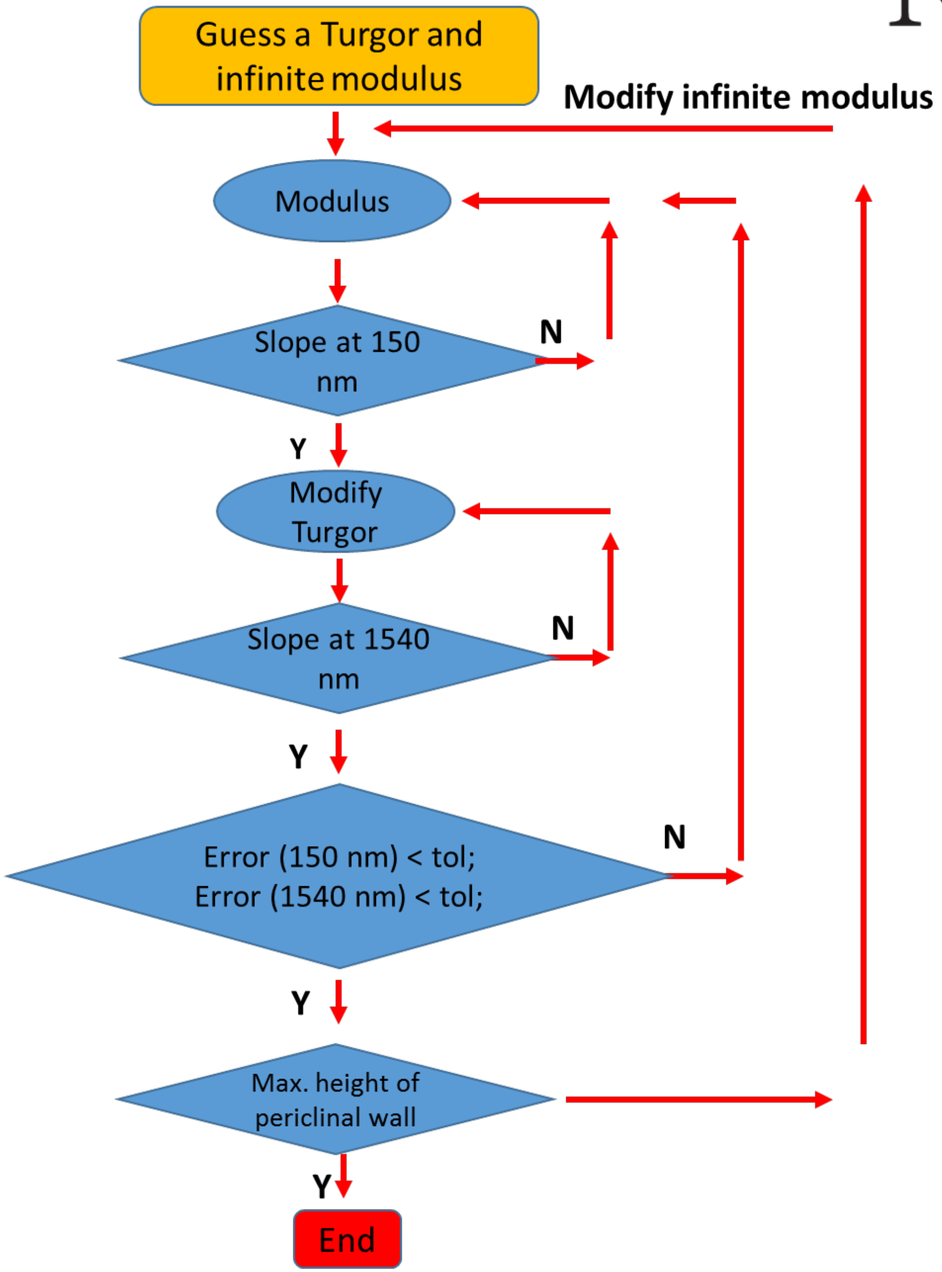


FE Model of the Nanoindentation Measurements



Iteration of FE Model

- Slope at shallow indentation depth is sensitive to instantaneous modulus (Forouzesh, 2013)
- Slope at deep indentation depth is most sensitive to turgor pressure
- The infinite modulus affects the static height of the pressurized periclinal wall, but has little influence on the indentation slope

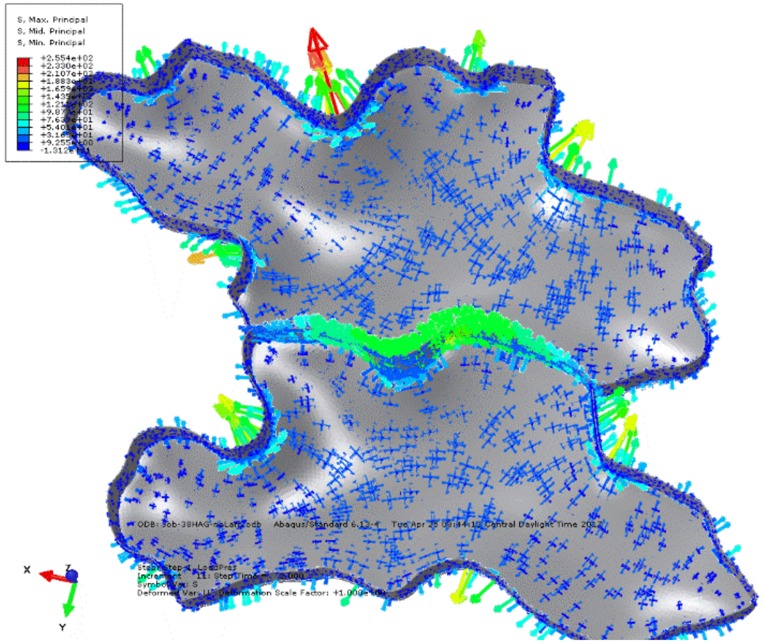


Mechanical Properties

- Mechanical properties govern the force-displacement relations
- Usually unknown for new materials and must be measured
- Nanoscale materials often behave differently than the same materials in macroscopic structure
- To measure these properties at the nanoscale the material must be mechanically deformed
- Time dependent properties are relevant for most polymers (viscoelasticity)

Other Measurements/Models

- Quantify the stresses and strains within groups of cells
- Plant cell wall failure
- Cell wall degradation
- Dynamic nanoindentation to quantify viscoelastic properties
- Measured differences with respect to age, genetics, cell type (i.e., guard cell vs. pavement cell vs. trichome)



Thank you!

Questions?

Pavement cell morphodynamics

<u>Genetics and multivariate live imaging</u>
Dan Szymanski, Dept. of Botany and Plant Pathology, Purdue U.

Samuel Belteton
Eileen Mallery
Makoto Yanagisawa

<u>Finite-element modeling and physical methods</u>
Joe Turner Dept. of Mechanical and Materials Engineering, U. Nebraska-Lincoln

Wenlong Li
Faezeh Afshar
Sedighe Keynia

Anastasia Desyatova (trichome)
Ehsan Rezaei (pavement cells)

<u>Lobefinder</u>
David Umulis (Purdue)
George Wu

<u>Funding</u>

NSF

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