

# **Atomic Force Microscopy Stiffness Tomography on Living *Arabidopsis thaliana* Cells Reveals the Mechanical Properties of Surface and Deep Cell-Wall Layers during Growth**

Ksenija Radotić,<sup>†</sup> Charles Roudit,<sup>§</sup> Jasna Simonović,<sup>†</sup> Patricia Hornitschek,<sup>¶</sup> Christian Fankhauser,<sup>¶</sup> Dragosav Mutavdžić,<sup>†</sup> Gabor Steinbach,<sup>||</sup> Giovanni Dietler,<sup>‡</sup> and Sandor Kasas<sup>‡§</sup>

<sup>†</sup>Institute for Multidisciplinary Research, University of Belgrade, Belgrade, Serbia; <sup>‡</sup>Laboratoire de Physique de la Matière Vivante, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland; <sup>§</sup>Département de Biologie Cellulaire et de Morphologie and <sup>¶</sup>Center for Integrative Genomics, Université de Lausanne, Lausanne, Switzerland; and <sup>||</sup>Hungarian Academy of Sciences, Biological Research Centre, Szeged, Hungary

## **Supporting Material**

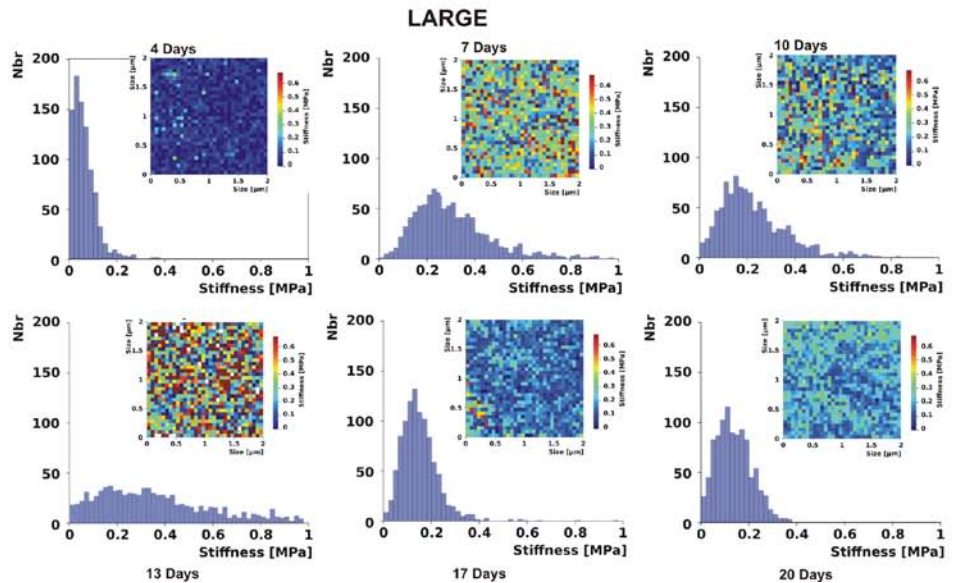
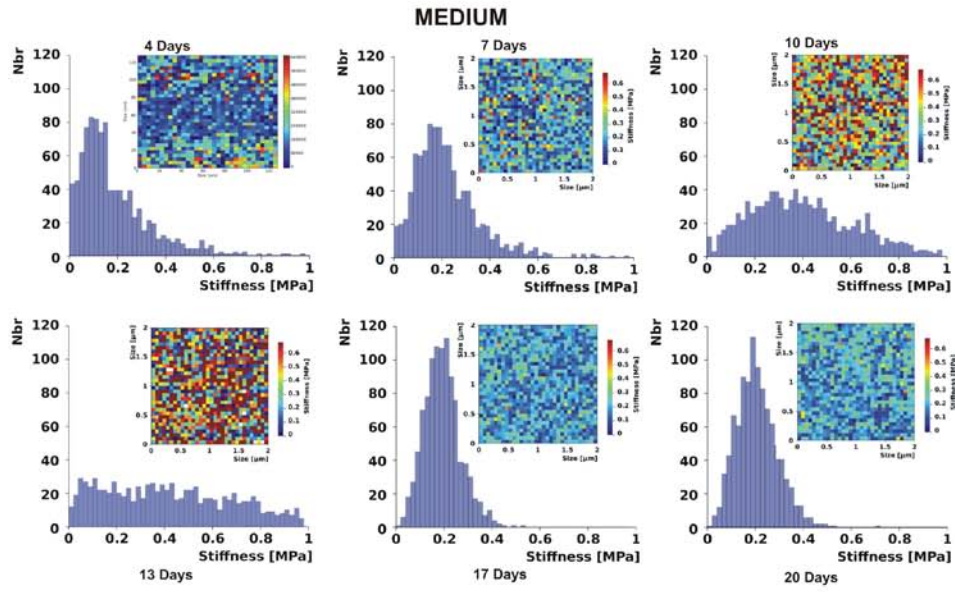
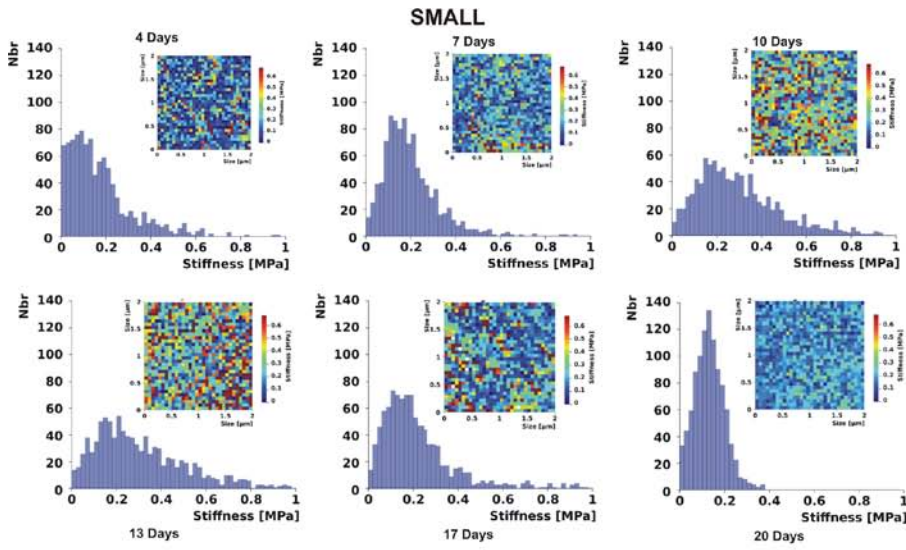
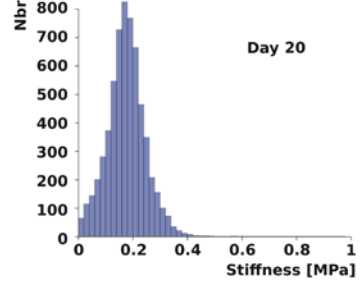
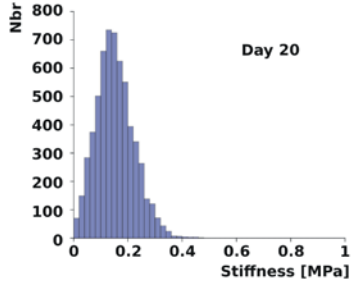
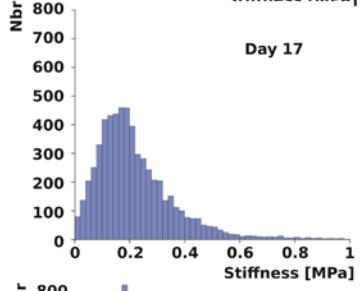
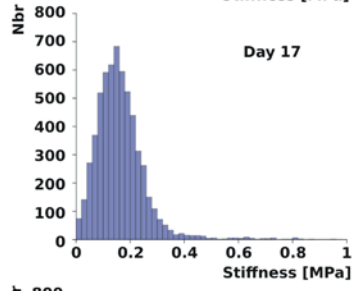
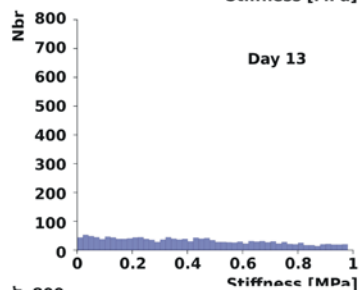
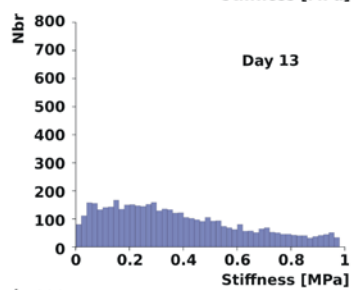
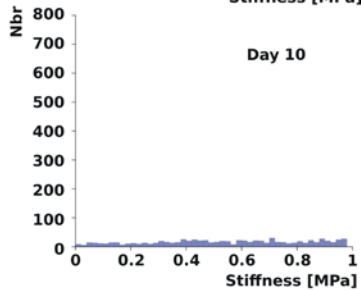
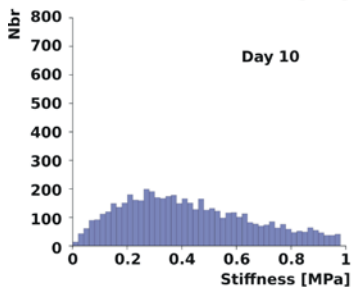
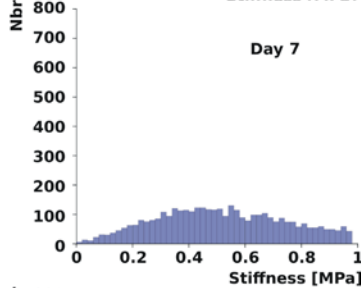
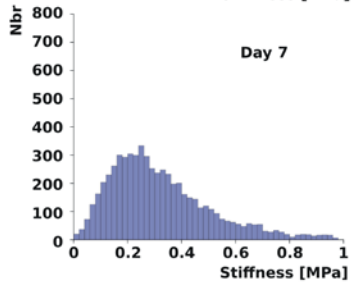
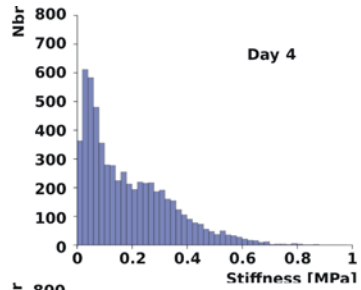
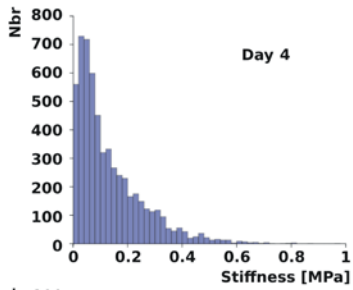


Figure S1. Cell-wall stiffness profiles of suspension-cultured cells of *Arabidopsis thaliana* down to an indentation depth of 10 nm after 4, 7, 10, 13, 17 and 20 days of cultivation, for the small (A), middle (B) and big (C) cells. The histograms are constructed from the surface-stiffness maps of two 2  $\mu\text{m}$  x 2  $\mu\text{m}$  areas recorded on 6 different cells in total. The insets correspond to a single 2  $\mu\text{m}$  x 2  $\mu\text{m}$  stiffness map arbitrarily chosen for illustration among the recorded data set.



10-20 nm depth

30-40 nm depth

Figure S2. The histograms constructed from the corresponding surface-stiffness maps, of suspension-cultured cells of *Arabidopsis thaliana* after 4, 7, 10, 13, 17 and 20 days of cultivation, for two different cell wall depths.

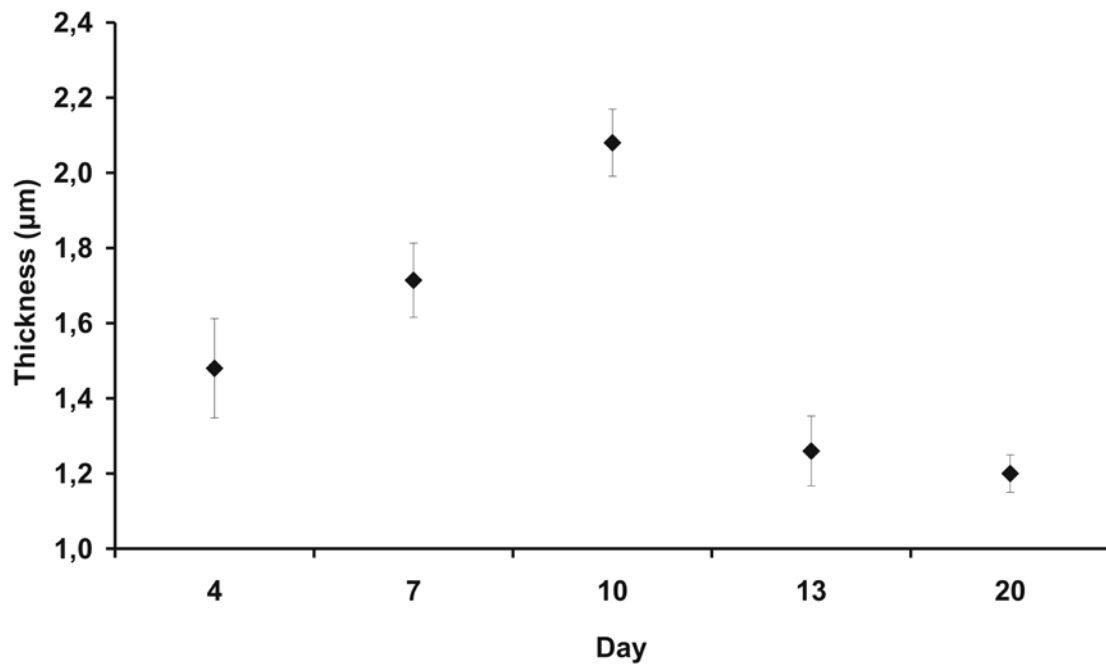


Figure S3. The average cell wall thickness ( $\pm$  SE) for different suspension ages. On the basis of Duncan test, the day 10 is significantly different from all the other points, while the day 7 is significantly different from all the other points except the 4 day.

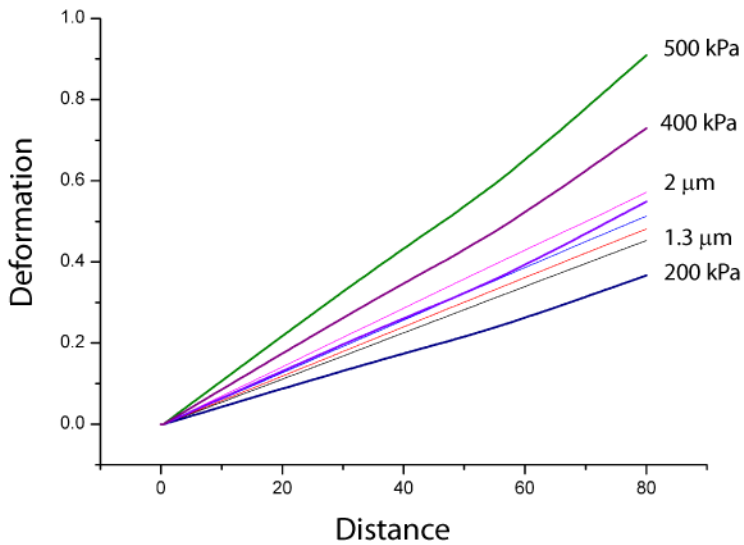
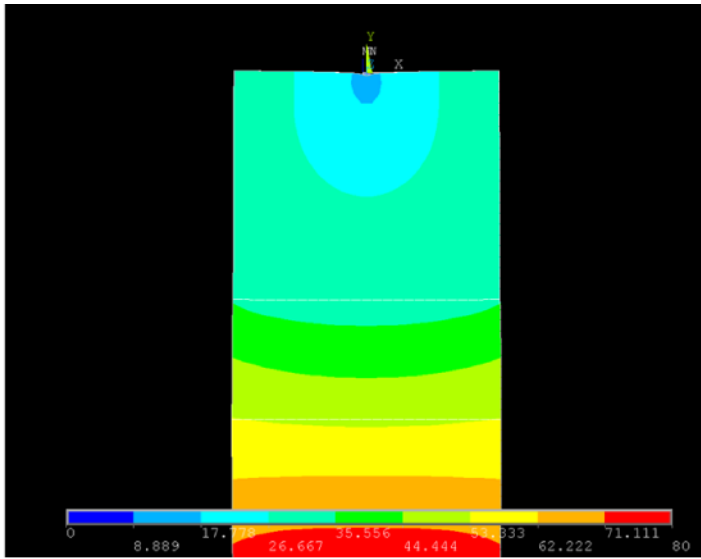


Figure S4. Upper panel: Finite element simulation of an AFM tip indenting a 1500 nm large and 3000 nm high sample. The tip having a radius of 20 nm is invisible on this image. False colors represent the absolute value of the total deformation vector; Lower panel: Finite element simulations result. Thick lines represent FD curves obtained on

“reference” samples having a Young’s moduli of 200, 300, 400 and 500 kPa (thick lines).

Thin lines represent force distance curves obtained on simulated cells having a 500kPa stiff cell wall with a thickness varying between 1.3 ,1.5, 1.7 and 2 microns.