DOI: 10.1017/S1431927605501855
Microsc Microanal 11(Suppl 2), 2005

A comparison of atomic force microscopy and field-emission scanning electron microscopy for imaging the plant cell wall

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The plant cell wall is a highly complex material. While the structures of the component macromolecules have been reasonably well elucidated, the overall architecture of the cell wall remains enigmatic. A common way to examine cell wall ultrastructure has been where the surface is exposed and a metal-carbon replica made and examined with transmission electron microscopy. More recently, cell wall surfaces have been imaged directly with field-emission scanning electron microscopy (FESEM), which removes the difficulty of handling a replica [1, 2]. However, FESEM requires the sample be dehydrated and critically point-dried, which may alter structure, and a metal coat is usually needed to generate sufficient contrast and avoid charging. To assess the ultrastructural alterations caused in the cell wall by FESEM, we compared cell walls prepared for FESEM to those imaged with atomic force microscopy (AFM). AFM has been used to investigate cell wall ultrastructure but mostly on cell walls that have been homogenized rather than in intact tissue [3, 4].

For material, from the hypocotyls of dark-grown cucumber (*Cucumis sativus*), we examined cortical parenchyma, which are large and relatively uniform cells, and in which the wall surface is exposed by bisecting the hypocotyl in water. Samples for AFM were imaged in air on Nanoscope III (Digital Instruments). Highest contrast images were obtained when the scan angle of the tip was at 45^{o} from the longitudinal axis of the hypocotyl. Measurements were performed in contact mode at a scan rate of 1 or 2 Hz. The AFM was fitted with silicon nitride triangular cantilevers (Sharp Microlever, Veeco, CA) having a nominal spring constant of 0.03N m^{-1}. Samples for FESEM were dehydrated in a graded ethanol series, critical-point dried, sputter coated with platinum (ca. 2 nm), and examined in a Hitachi S4700 cold-cathode field-emission scanning electron microscope at 5 kV, with working distance between 5 to 7 mm.

FESEM produced images where the microfibrils undulated gently and formed a solid mat (Fig. 1A). The diameter of microfibrils appeared to vary continuously, which may reflect different extent of encrusting matrix materials. Structures down to about 5 nm could be resolved. AFM of cell walls that were moist (i.e., imaged in air but immediately after removal from water) revealed a similar structure, albeit at lower resolution (Fig 1B). Microfibril diameter appeared larger in AFM. AFM images were produced of samples after fixation, dehydration to 50% ethanol, and after sputter coating. No disruption of structure was evident.

To confirm the visual impression of unaltered structure, we used a novel algorithm for measuring the shape of the Fourier transform as a function of frequency. Transforms at all frequencies were elliptical and the ellipticity was a function of frequency, indicating the different levels of orientational order at different frequencies. However, the transforms of the treated samples were indistinguishable from the untreated ones, indicating that the cell wall is robust to the preparation treatment and validating the use of FESEM for cell wall ultrastructural analysis.

We are using both FESEM and AFM to analyze the changes in cell wall ultrastructure that occur during plant cell growth [5].

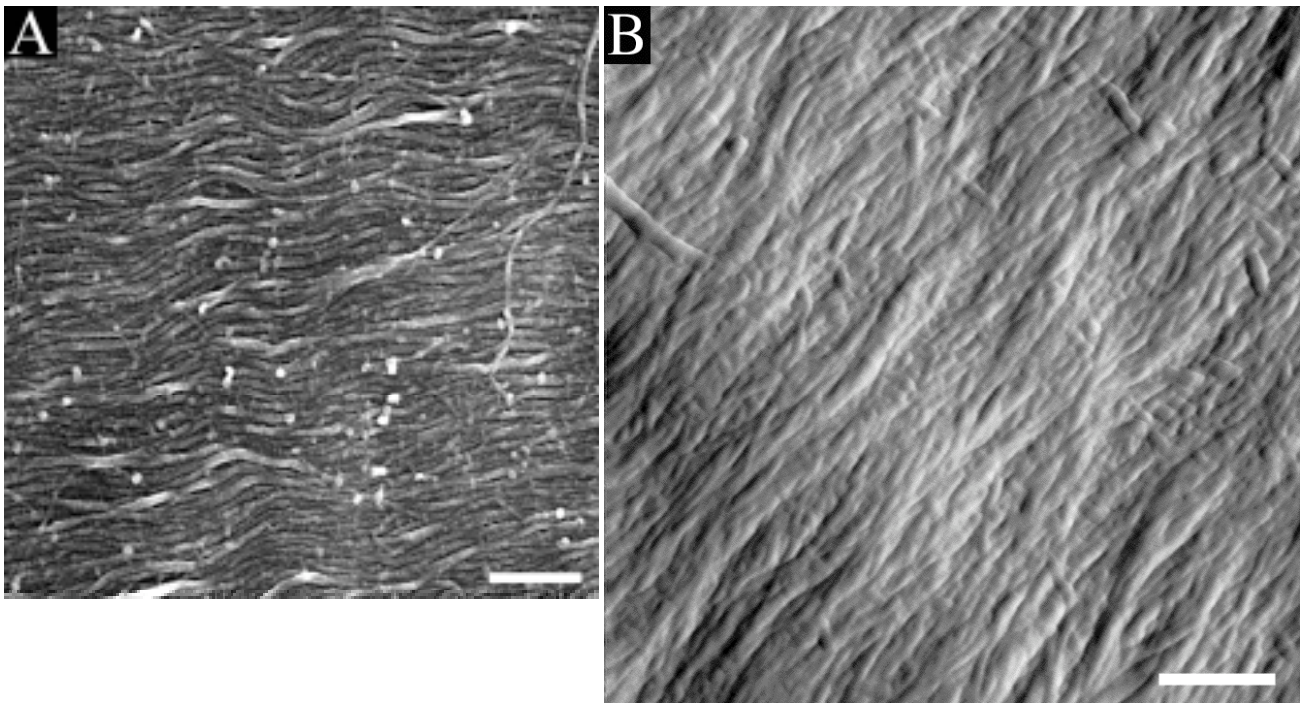

Figure 1. Micrographs of the innermost surface of the cell wall cortical parenchyma in cucumber hypocotyls. The long axis of the hypocotyl is parallel to the long axis of the page for A and at 45˚ for B. **A**. FESEM image. Sample was bisected in water to remove cytoplasm, fixed, dehydrated, critically point dried, sputter coated with Pt, and imaged. Bar = 200 nm. **B**. AFM image. Sample was bisected in water and imaged immediately. Bar = 250 nm. Panel B has been scaled to make both panels have roughly the same magnification. Overall structure is similar in the two images except that the apparent diameter of the microfibrils in the AFM image is larger, presumably because they are somewhat compliant in the AFM.

References

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[5] We thank Daniel Cosgrove (Penn State University) for the collaborative cucumbers. This work was supported in part by a grant from the US Department of Energy to TIB (award No. 03ER15421), which does not constitute endorsement of that department of the views expressed herein.