Three-dimensional water mapping of succulent *Agave victoriæ-reginæ* leaves by terahertz imaging

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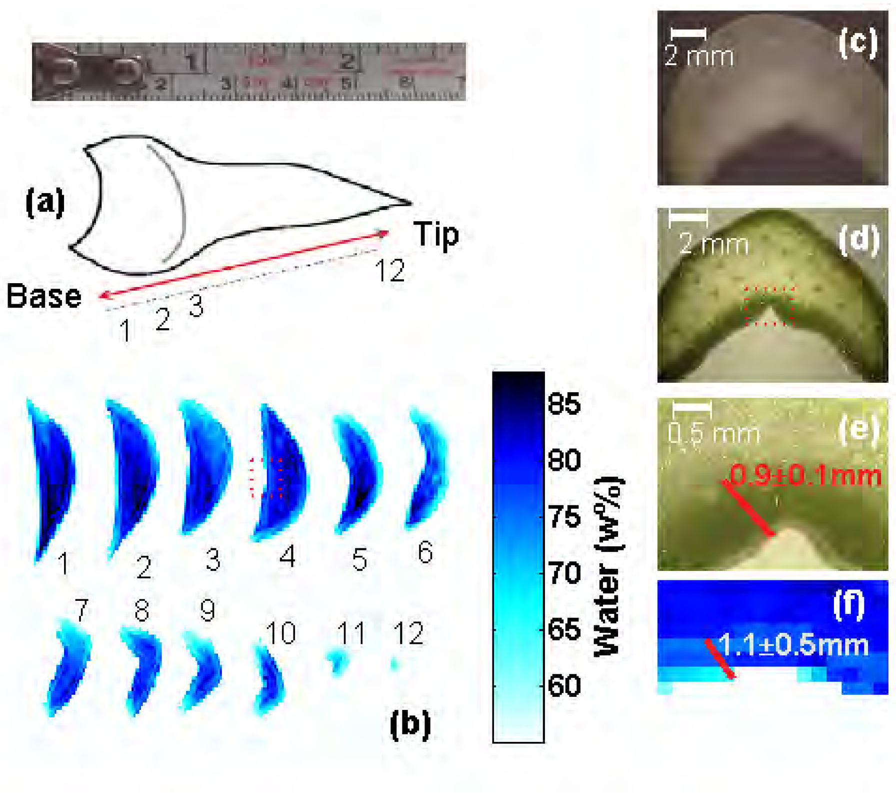
We present the first three-dimensional water mapping of an agave leaf, employing terahertz time-domain spectroscopic imaging, which demonstrates an unprecedented capability to study the water retention mechanisms within succulent plants. We found that agave leaves are composed of a low-hydration outer tissue layer, defined by the outermost layer of vascular tissue that surrounds a high-hydration tissue, the carbohydrate rich hydrenchyma. The findings are supported by histological images and the correlation between the water content and carbohydrate presence is consistent with recently published findings of a remarkably large hydration shell associated with agave fructans.

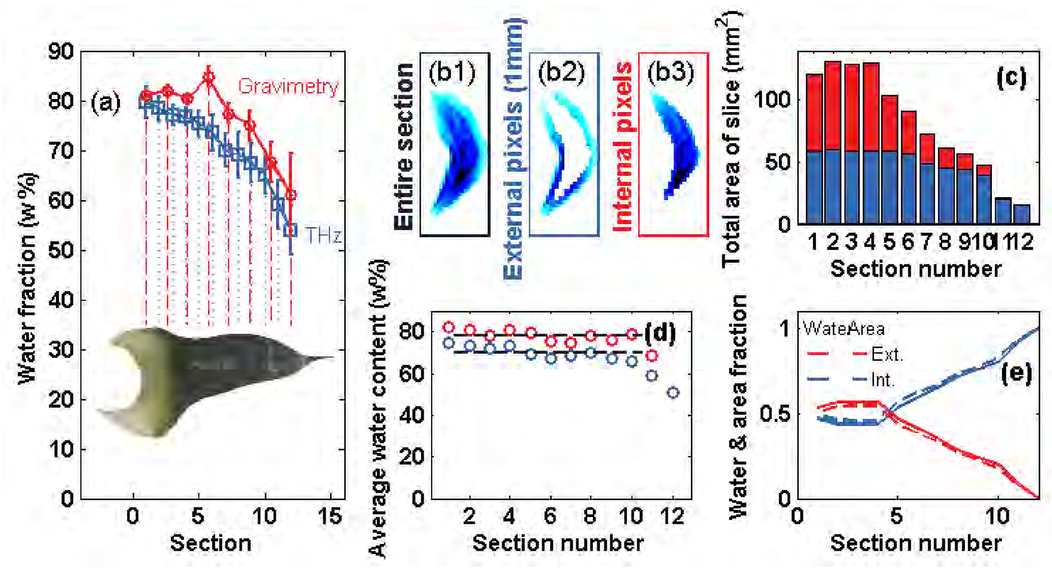
Terahertz Imaging: Water content in leaf sections

Given the fact that water is a highly absorptive liquid at terahertz frequencies, the transmitted intensity of the terahertz radiation is a function of the water content of the plant tissue. Since the agave leaves are significantly thicker than the leaves from typical plants, we performed measurements on thin 0.6 mm transverse sections of the agave leaves.



Determination of water content in leaf sections of *A. victoriæ-reginæ* leaves.(a) Approximate positions of sections 1-12 over the length of an *A. victoriæ-reginæ* leaf (b) Tomographic sections showing the water content distribution of an *A. victoriæ-reginæ* leaf. (c) Optical microscopy images of an *A. victoriæ-reginæ* basal (white) leaf section at 8X. (d,e) Bright light microscopy images of an *A. victoriæ-reginæ* middle (green) leaf section at 8X and 35X magnification respectively. The red box in (d), indicates the enlarged region shown in (e). (f) Enlarged terahertz image corresponding the box area of section 4 in (b)

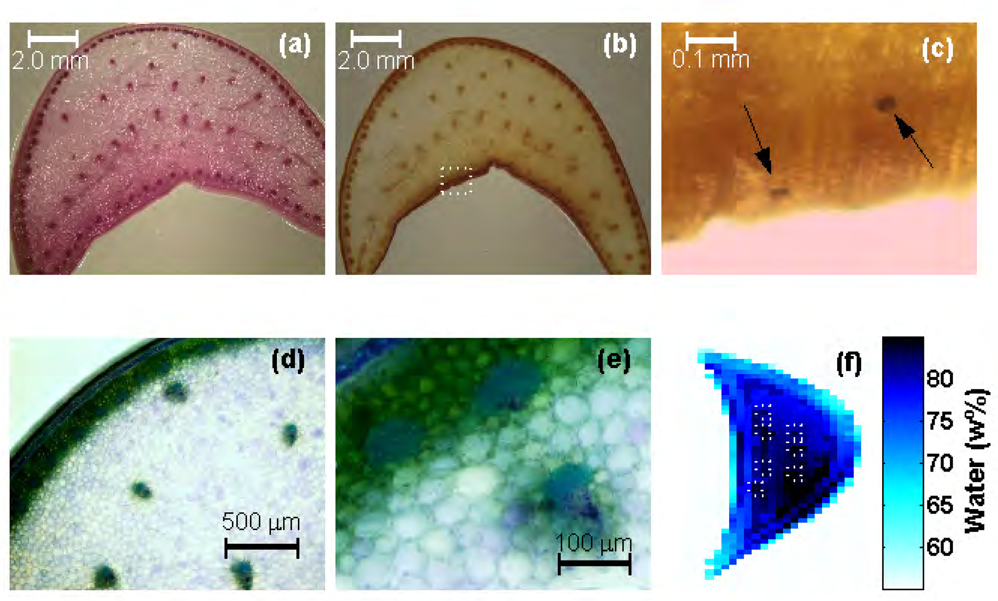
Terahertz Spectroscopy vs Gravimetry



(a) Average water content for each leaf section of *A. victoriæ-reginæ*, comparing the determination of water by traditional methods (red) and by terahertz imaging (blue). (b1) Water mapping of a single section, the pixels in the external 1 mm layer (b2) and the pixels in the internal part (b3) respectively. (c) Area of each section along the leaf separating the contribution of the external, 1 mm layer (blue) and the internal pixels (red). (d) Water content of the external (blue) and internal (red) pixels. (e) Fraction ofwater of each section that corresponds to the external (continuous blue) and internal (continuous red) pixels, and fraction of the total area (dotted lines).

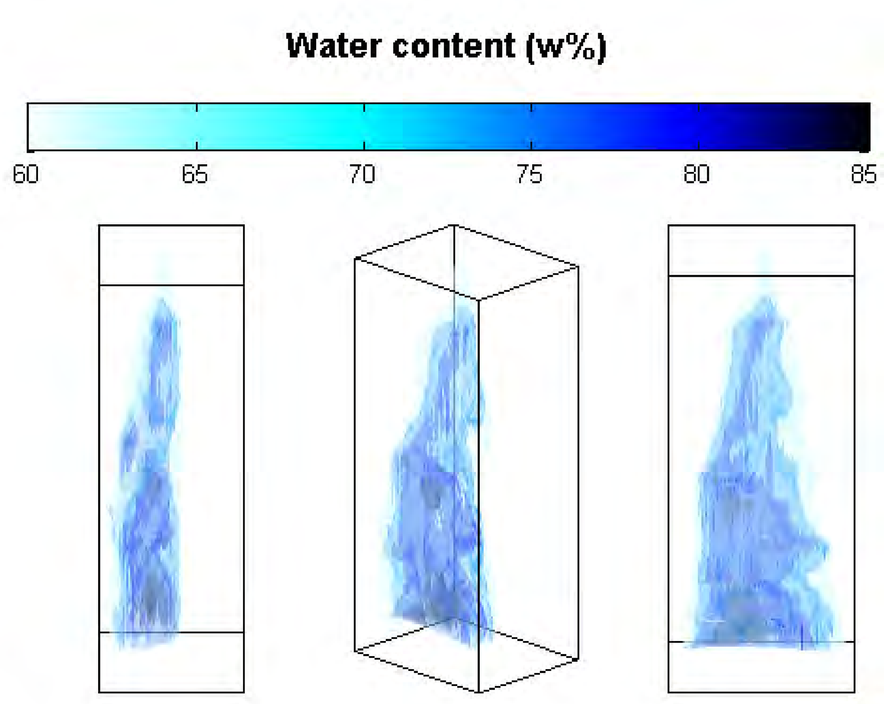
Carbohydrate Staining

In order to explore the association between carbohydrates and water content in *A. victoriæ-reginæ* leaves, transverse sections of white basal leaf tissue were stained by either PAS (an non-specific stain for carbohydrates) or lugol (a specific stain for starch).



Carbohydrate staining of *A. victoriæ-reginæ* leaf cross sections.(a) Micrograph 8X of a leaf section after PAS staining. The pink coloring correlates with the presence of total carbohydrates. (b) and (c) are 8X and 35X micrographs of lugol stained sections for starch identification. Panels (d) and (e) contain micrographs, highlighting the vasculature of the leafs. In (f) closeup of a section terahertz image of a single slice is shown, boxes were placed in order to identify areas of higher hydration that correlate with the position of the veins on the micrographs.

Three-dimensional Water Mapping



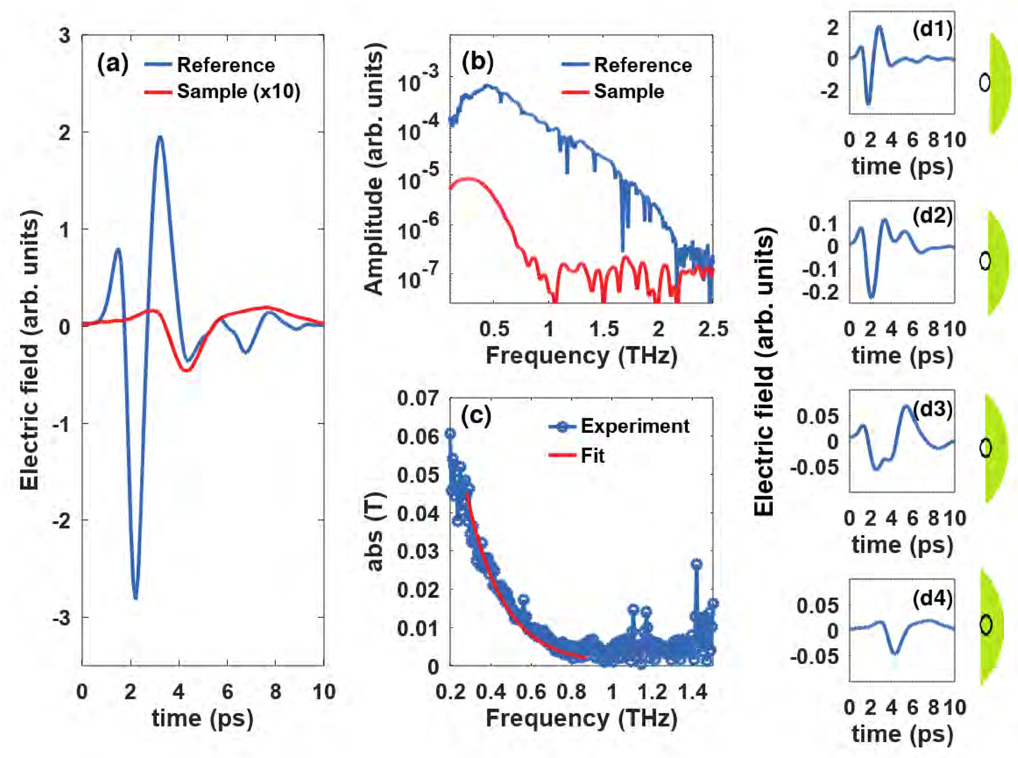
3-Dimensional view of the water distribution in an *Agave victoriæ-reginæ* leaf. The image shows a relatively thin layer of low (<70 w%) water content tissue in the outside, pale blue, surrounding the more succulent part that appears in darker colors (>74 w%). The representation was generated by superimposing the 62w %, 74 w%, 80 w% and 85 w% translucent isosurfaces. The animation can be seen in the PDF version of the article using Acrobat Reader or in http://www.thz.org.mx/MuestrasIrapuato.php featuring details of a reconstructed image using THz spectroscopy.

Image Processing

In order to process the terahertz images and estimate the pixel-by-pixel water content, we use Landau-Lifhitz-Looyenga effective medium theory model expressed as

$$\sqrt[3]{\epsilon_L(\omega)} = x_W\sqrt[3]{\epsilon_W(\omega)} + x_S\sqrt[3]{\epsilon_S(\omega)} + x_A\sqrt[3]{\epsilon_A(\omega)}, \quad (1)$$

where ϵ_k are the dielectric functions of the mixture components and x_k are the relative volumetric fractions. The indices refer to leaf (L), water (W), solid/dry tissue (S) and air (A), respectively, and ω is the frequency.



(a) Terahertz time-domain reference signal and the signal transmitted through hydrated agave slice; (b) Frequency domain electric field amplitude of reference signal and signal transmitted through hydrated slice; (c) Experimental values of the transmission amplitude (blue dots) and fit using the model (red line). (d1-4) Terahertz waveforms measured passing through the pinhole while scanning across the edge of the leaf. The signals (d1) and (d4) corresponds positions where pinhole is either completely outside the sample or completely inside the sample, respectively. The signals (d2) and (d3) are positions where the signal was partially transmitted through the sample and partly through air. The schematics shown on the right of each subfigure depict the position of the pinhole for each measurement. In order to prevent inaccurate measurements all pixels that did not comply with full transmission through the sample were discarded.

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References

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