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Laser Ablation Tomography (LATscan): Enabling Natural Color 3D Microanalysis of Biological Systems

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Enabling Natural Color 3D Microanalysis of Biological Systems

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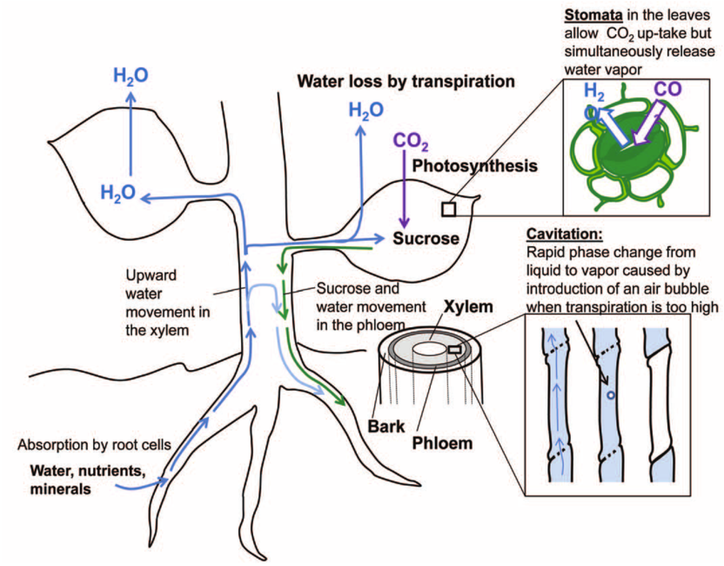
Abstract

Laser ablation tomography (LATscan) is a new 3D structural analysis technique that provides researchers with data they did not have access to before. LATscan is a faster, higher resolution, material agnostic method that promises to fundamentally improve specimen analysis from medical research to agrochemical research. The technology uses an ultraviolet (UV) pulsed laser is used to continually ablate thin layers of samples while simultaneously imaging them. These images perfectly capture the cross-sections in their natural state prior to ablation. The color resulting from UV-induced fluorescence in the images allows for easy image segmentation to identify microscale anatomical and compositional features, and thus samples do not need staining. The images are then stacked to produce 3D models for volumetric microanalysis. The image resolution of the sections is comparable to optical microscopy modalities, and the distance between slices is only limited by the resolution of the stage feeding the sample into the laser ablation plane. This poster explains the technology, and presents results from a use-case study on soybean stems. Quantifying vascular features in soybean stems will allow researchers to better understand the relationship between genetics and drought-resilient crop. This understanding will result in water-efficient soybean crops that can grow in diverse environments.



Introduction

Genetically modified soybeans aim to alter properties of the xylem to improve hydraulic functions to create drought resilient crop. Which allows them to grow in more diverse environment. This enhancement of the soybean will potentially increase the number of xylems, in particular the metaxylem vessels that are within the soybean. The increase will improve the stomatal conductance and root hydraulic conductivity resulting in a higher yields from longer lasting crop. Modifying the soybean's root system will significantly increase crop productivity and crop yield, which in turn will act as a catalyst to a new green revolution, solving the growing demand for food. With the improvement and innovation of laser ablation tomography (LATscan), new and never-before seen imaging and modeling can be produced, allowing for more in-depth research to be done on the xylem and its effects on the hydraulic system. The use of the LATscan can extract new data suggesting that the increase number of metaxylem in a soybean can vastly decrease the flux of water with in a soybean, resulting in a more water efficient soybean. LATscan lets us look at a close up of the cross section of the plant, helping to identify phenotypic alterations being engineering by crop scientists..

Acknowledgements

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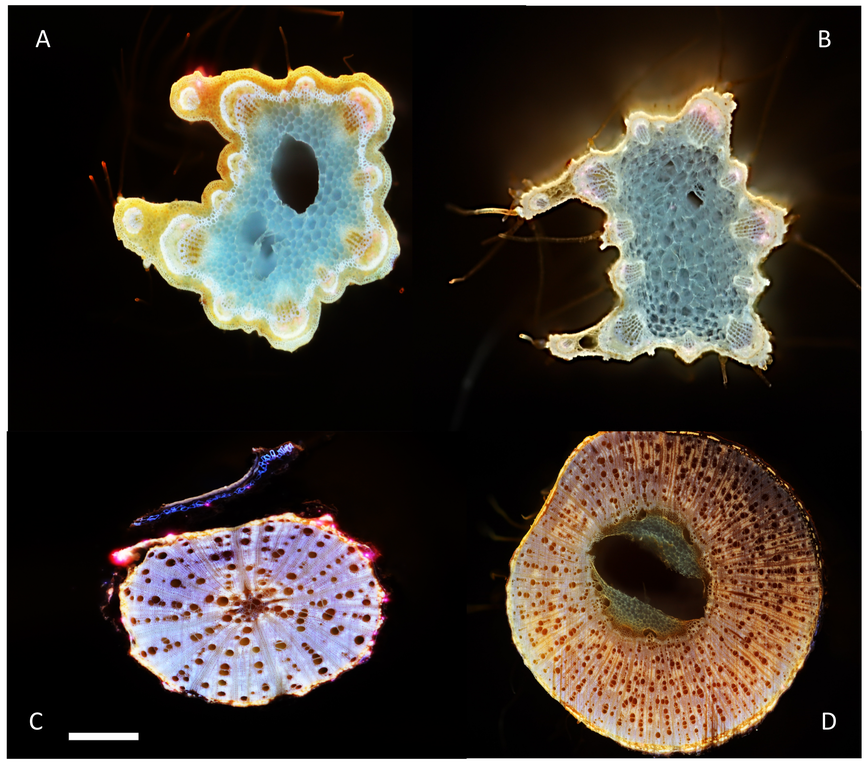
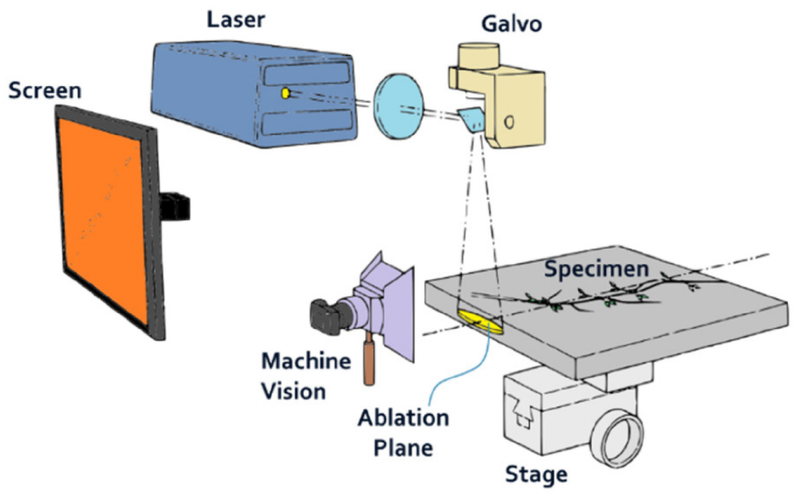
Developed with LAME

Prince, Silvas J., et al. "Root Xylem Plasticity to Improve Water Use and Yield in Water-Stressed Soybean." *Journal of Experimental Botany*, July 2017, doi:10.1093/jxb/erw472.
Hall, Benjamin, and Asheesh Lanba. "Three-Dimensional Analysis of Biological Systems via a Novel Laser Ablation Technique." *Journal of Laser Applications*, vol. 31, no. 2, 2019, p. 022602., doi:10.2351/1.5096089.



Above: Images of Soy bean plant stem [D], root [C], and peliole [A,B] created with LATscan technology. Scale bar is 500 microns

Below: Schematic showing the setup of a LATscan system and actual set up.



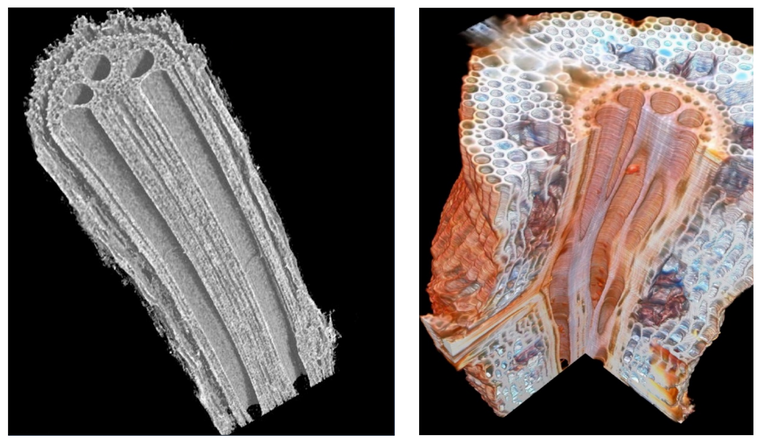
Methods

An Avia 355-7000 Q-switched ultraviolet laser source is used with a pulse repetition rate between 25 and 40 kHz. The wavelength for this ultraviolet laser is 355 nm. The pulse duration of the laser is less than 30ns and supplied a pulse energy of approximately 150–200 µJ. The laser is coupled with a Scanlab HurryScan 10 galvanometer to rapidly scan the beam along a line, effectively creating a "laser sheet," which is refer to as the ablation plane. Then the sample is moved into the ablation plane, an Anorad servo driven stage with 300 mm of travel. The stage is controlled with a Galil motion control software, which can either be incremented at preset intervals or continuously traversed at a defined velocity, coupled with a set image capture frequency to achieve a desired z-step resolution. The stage velocity is set at 10 µm/s creating the perfect frame rate. A Canon 70D equipped with a Canon Macro Photo Lens is used to capture the images and later put into FIJI for further analysis.

Comparing X-ray Microtomography and LATscan

X-ray Microtomography

LATscan



Left: analysis of sample performed on GE Nanotom microCT ~approx 2 hrs acquisition time. Right: LAT scan of fresh sample ~40 second data acquisition time. LATscan has a naturally colored image and are able to identify more xylem. The roots are approximately 2-3 mm wide

Conclusions

With the innovation of LATscan, a new ability to comprehend the effect of phenotyping of soybean's xylem structure and the overall plant structure with a full 3D image/model has emerged. The increase of xylem with the influence hydraulic function will overall change the soybeans ability to adapt to dry environments. Along with the increase of crop percent yield will ultimately create economical growth. All of this is do to the large advantage given by the LATscan's amazing ability to quantify information that would never have been seen, towering over previous methods used in the field. LATscan is a gateway to new and better ideas creating a brighter future.