Microneedle Sensors for *in situ* Measurements in Sorghum

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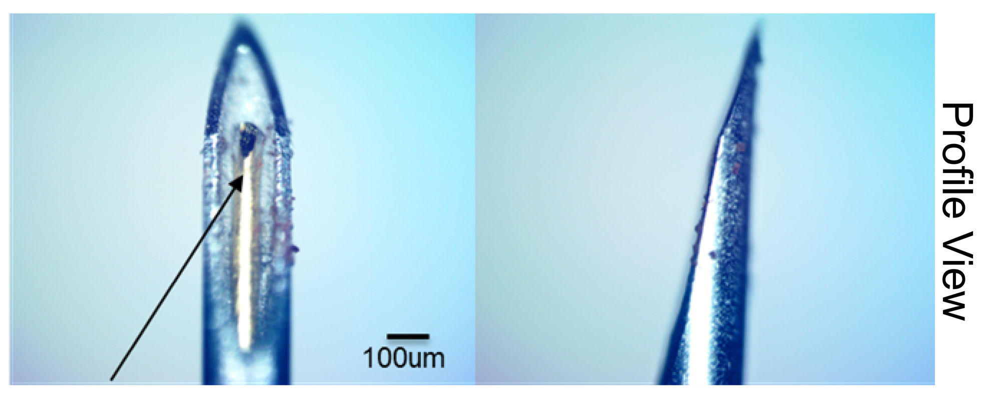
Coaxial Microneedle Sensor Design

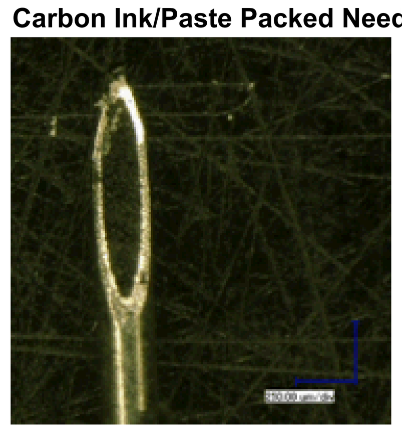
PEN Needles w/Au Microwire



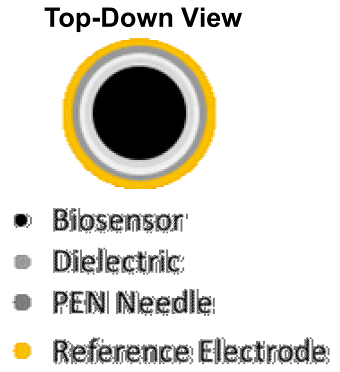
Electrically insulated 50um Au wire

- Microneedle technology is being explored for minimally invasive plant metabolite detection and as a fieldable sensing platform
- Created method for protecting electrode within needle
- Can be used for reference and/or working electrode
- Wire electrically isolated from needle w/e-coat
- Each needle is a sensor and large arrays of individually addressable sensor possible

Carbon Ink/Paste Packed Needle



Schematic of Coaxial Microneedle Top-Down View

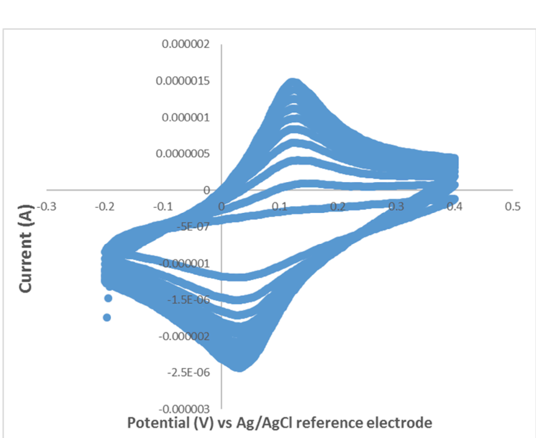


- Coaxial microneedle sensors are designed to place the sensor as close to the source as possible while using the needle to protect the sensor
- Interior biosensor can be either a wire or paste/ink, which can offer a more straightforward synthesis of the enzymatic sensors.
- Reference and counter electrode made with same fabrication methodology
- Puncture tests in sorghum will determine optimal electrode material

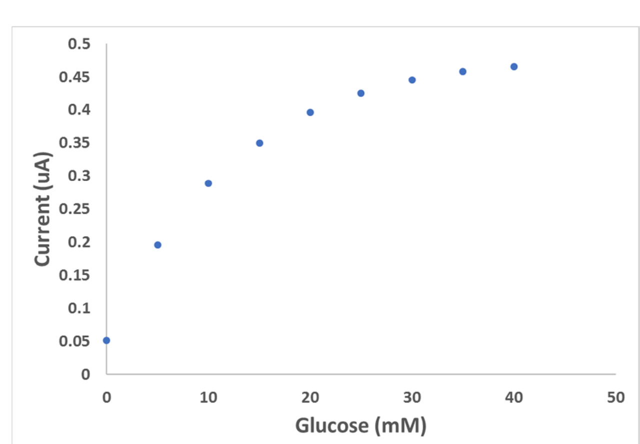
Carbon Ink-Packed Microneedle Glucose Sensor

The carbon ink coaxial microneedle glucose sensor was tested in solution across a concentration range based on the literature cited values of glucose in sorghum leaves (1-40mM). We suspect hole punch measurements average the glucose concentration across the leaf and that values in the phloem will be much larger and future experiments will test a serial dilution across a wider range of concentrations.

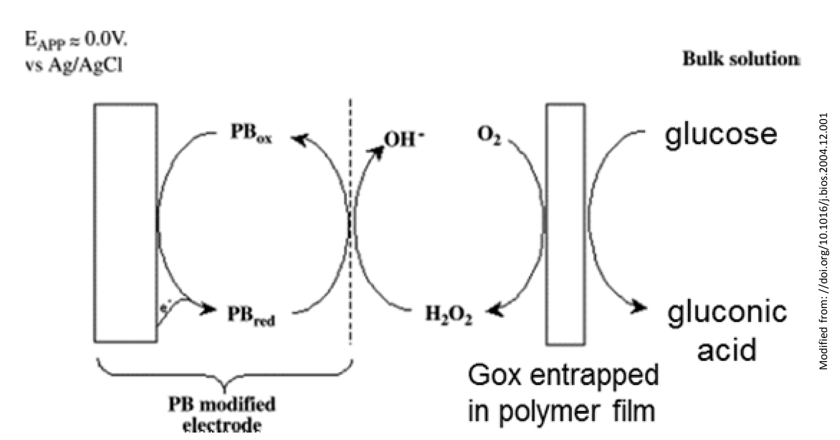
Detection of glucose was performed by a multi layer enzymatic biosensor. In the first layer, a mediator (prussian blue (PB)) was electrochemically deposited (left plot showing layer growth) on the carbon ink working electrode that acts as a catalyst for hydrogen peroxide reduction, which is a by-product of the glucose oxidase (enzyme) glucose interaction. The outer layer is an electrochemically polymerized polymer (polyphenylenediamine) to entrap glucose oxidase.



Electrochemical Deposition of Prussian Blue Layer



Glucose Detection Across Relevant Sorghum Range

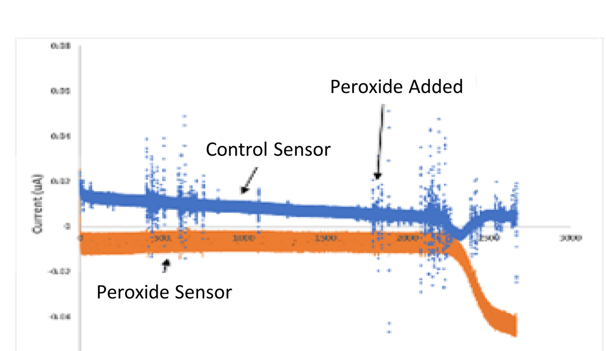


Electrochemical Detection Cascade

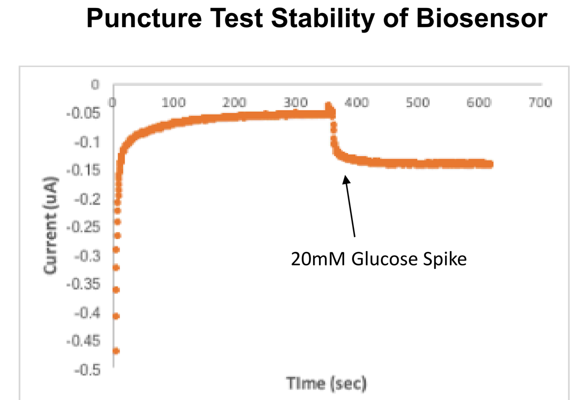
- Initial in-situ detection experiments of the coaxial needle peroxide sensor layer were performed in celery due to its ability to wick solution through its veins.
- Sensors were allowed to baseline prior to addition of peroxide to solution and delayed response on the functionalized sensor was seen in 7min.
- Typical response times of the sensor in solution are <20sec.
- Puncture test of glucose microneedle biosensor were performed in sorghum leaves with in solution tests following insertion to determine viability of enzymatic film.

Multiplexed Test Setup

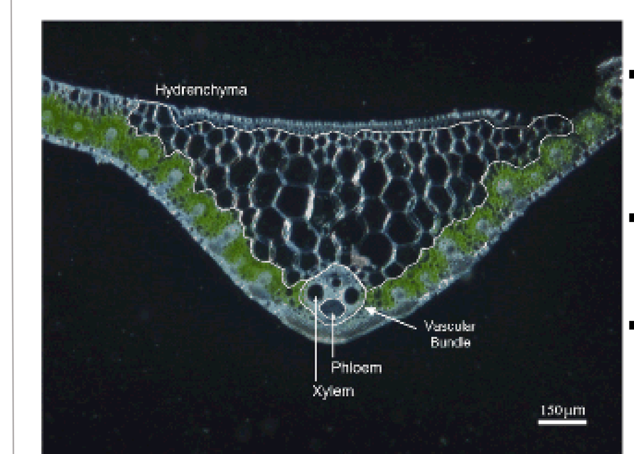
Multiplexed Detection



Puncture Test Stability of Biosensor



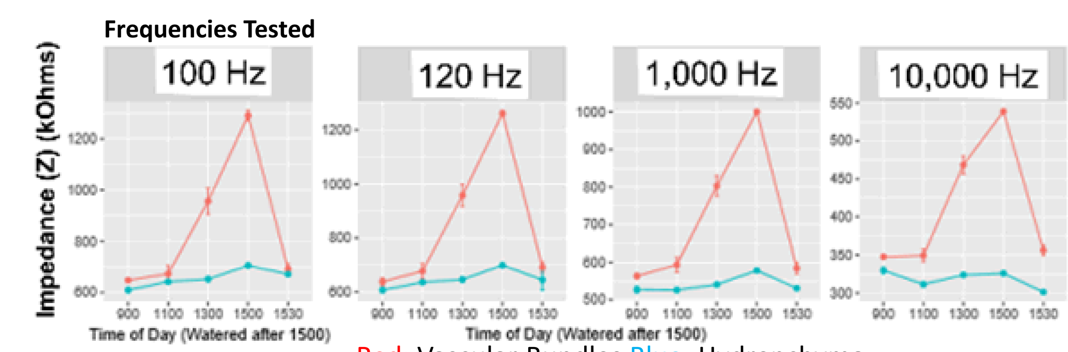
Targeted Tissue Impedance and Metabolite Sensing



Cross-section of Leaf Stem

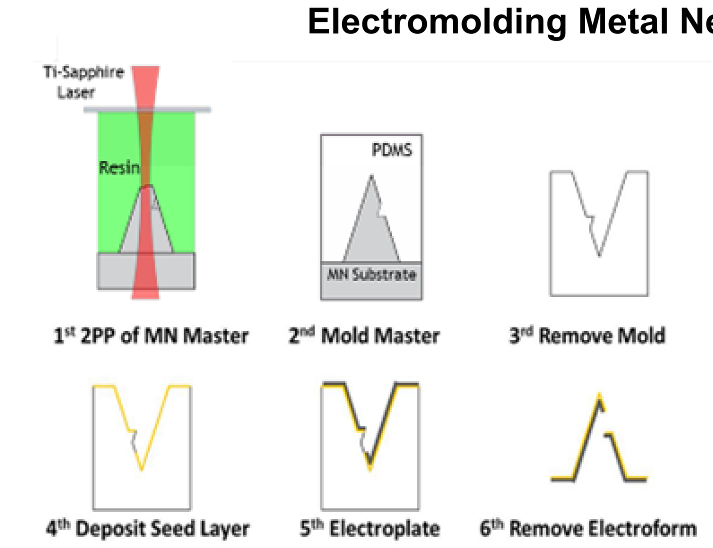
- Electromolded microneedles were used to probe the adaxial (AD) and abaxial (AB) surfaces of sorghum leaves during a short-duration drought experiment.
- AD-to-AD and AB-to-AB impedance measurements were made to measure the vascular bundles and the hydrenchama
- Preliminary results indicate an impedance increase in the vascular bundles while the hydrenchama remained relatively stable.

Frequencies Tested

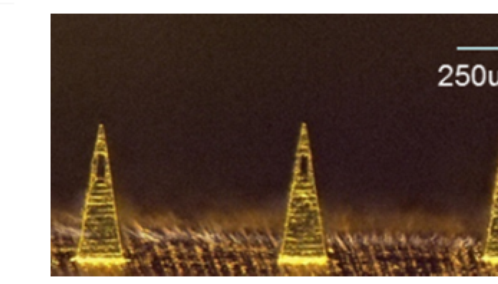


Red: Vascular Bundles Blue: Hydrenchyma

Electromolding Metal Needle Fabrication Method



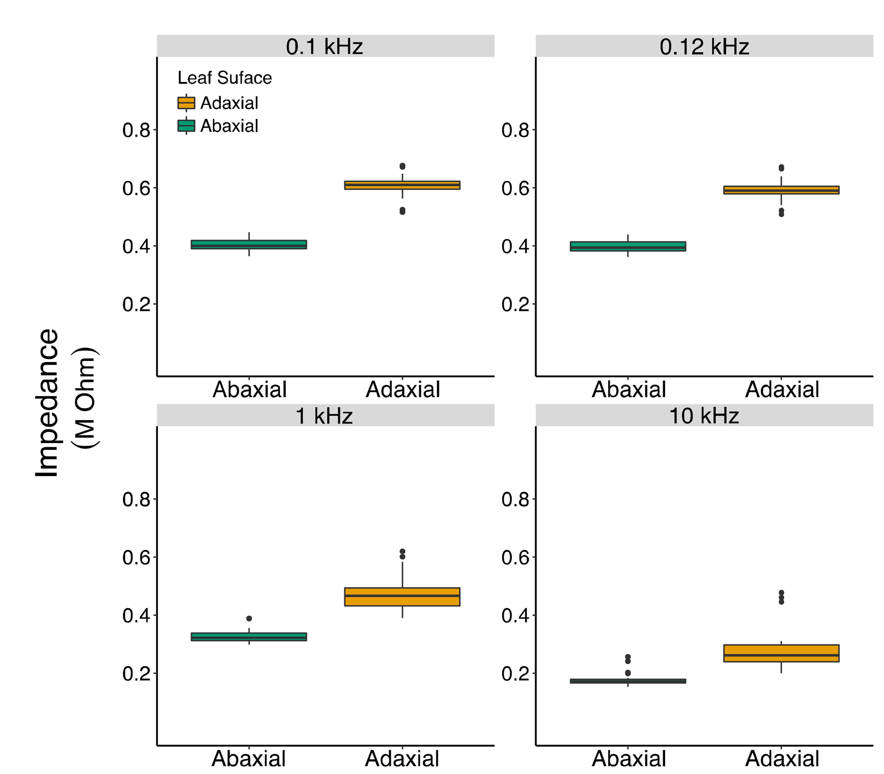
Fabrication schematic



- Existing scalable technology
- Conducting and hollow so can be used for each sensor system
- Proven strength for insertion in skin
- Complete control over geometry and bore placement
- Patent pending fabrication method

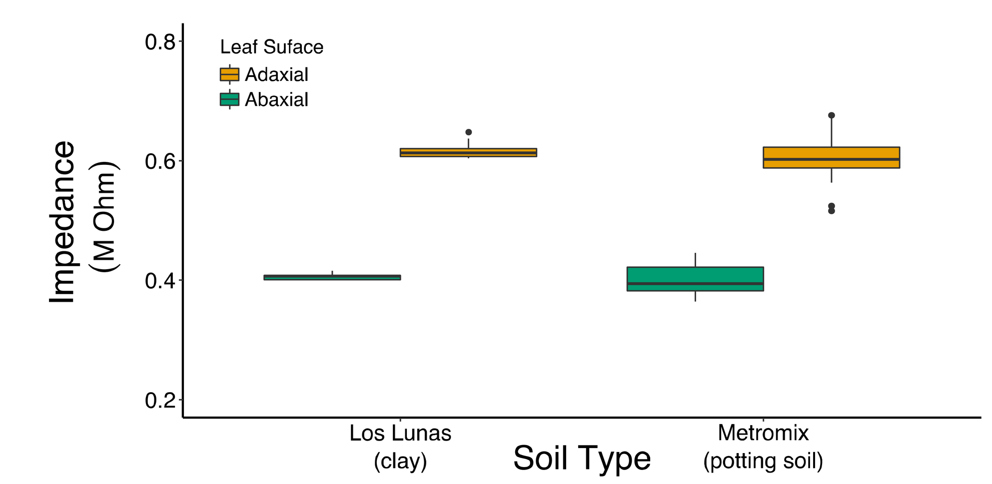
Impedance Variance Between Abaxial and Adaxial Surfaces.

- Transmitter located in leaf, receiver located in stem.
- Impedance sensor can distinguish distinct tissue types within a leaf.
- Vascular tissue has significantly lower impedance compared to hydrenchyma across all frequencies ($p < 0.0001$ in all comparisons).

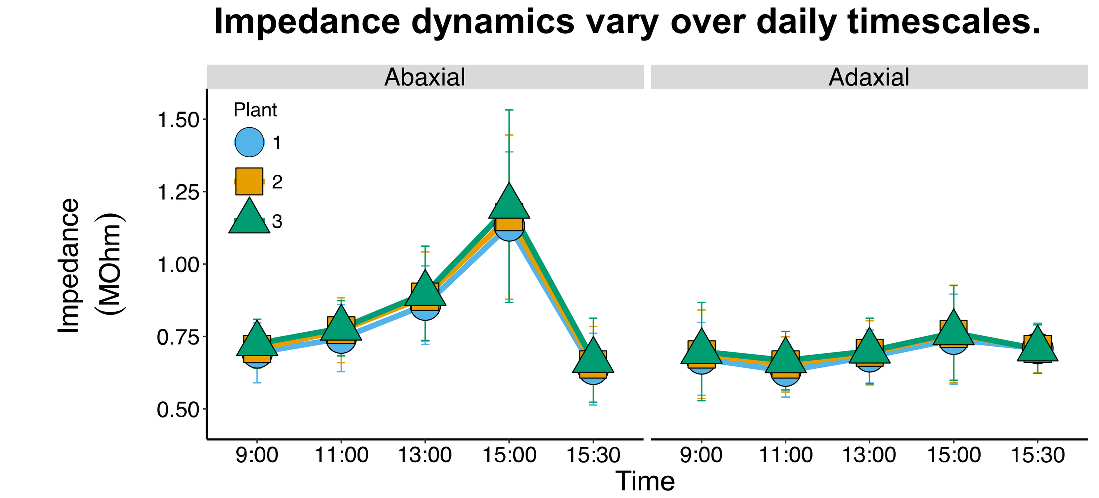


Impedance Variance Between Soil Type.

- Transmitter located in leaf, receiver located in stem.
- EIS data remained consistent between plants grown in clay and plants grown in potting soil.
- Our novel technique eliminates confounding influence of soil matrix on EIS measurements by restricting current transduction to within the plant body.



Impedance dynamics vary over daily timescales.



- Transmitter and receiver placed in leaf.
- Impedance sensor identified contrasting dynamics in tissue impedance.
- In non-stressed plants, vascular tissue exhibits larger fluxes in ion concentration than hydrenchyma.
- Late day water addition (15:00) increased apoplastic ion concentration in vascular tissue.

Future Directions

- Extend the duration of the impedance measurements and attempt to correlate the impedance values with water potential.
- Sucrose will be added to the sensor suite for multiplexed sensing of glucose and sucrose.
- Increasing the tissue impedance sample size of the drought experiments, testing the effect of different soils on measurements,
- Building a portable microimpedance analyzer to begin taking data in the field and at a larger scale.

References

1. Miller, Philip R., Roger J. Narayan, and Ronen Polsky. "Microneedle-based sensors for medical diagnosis." Journal of Materials Chemistry B (2016).
2. Miller, Philip R, et al. "Towards an Integrated Microneedle Total Analysis Chip for Protein Detection." Electroanalysis (2016).
3. Miller, Philip R, et al. "Multiplexed Microneedle-based Biosensor Array for Characterization of Metabolic Acidosis". Talanta (2011).

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