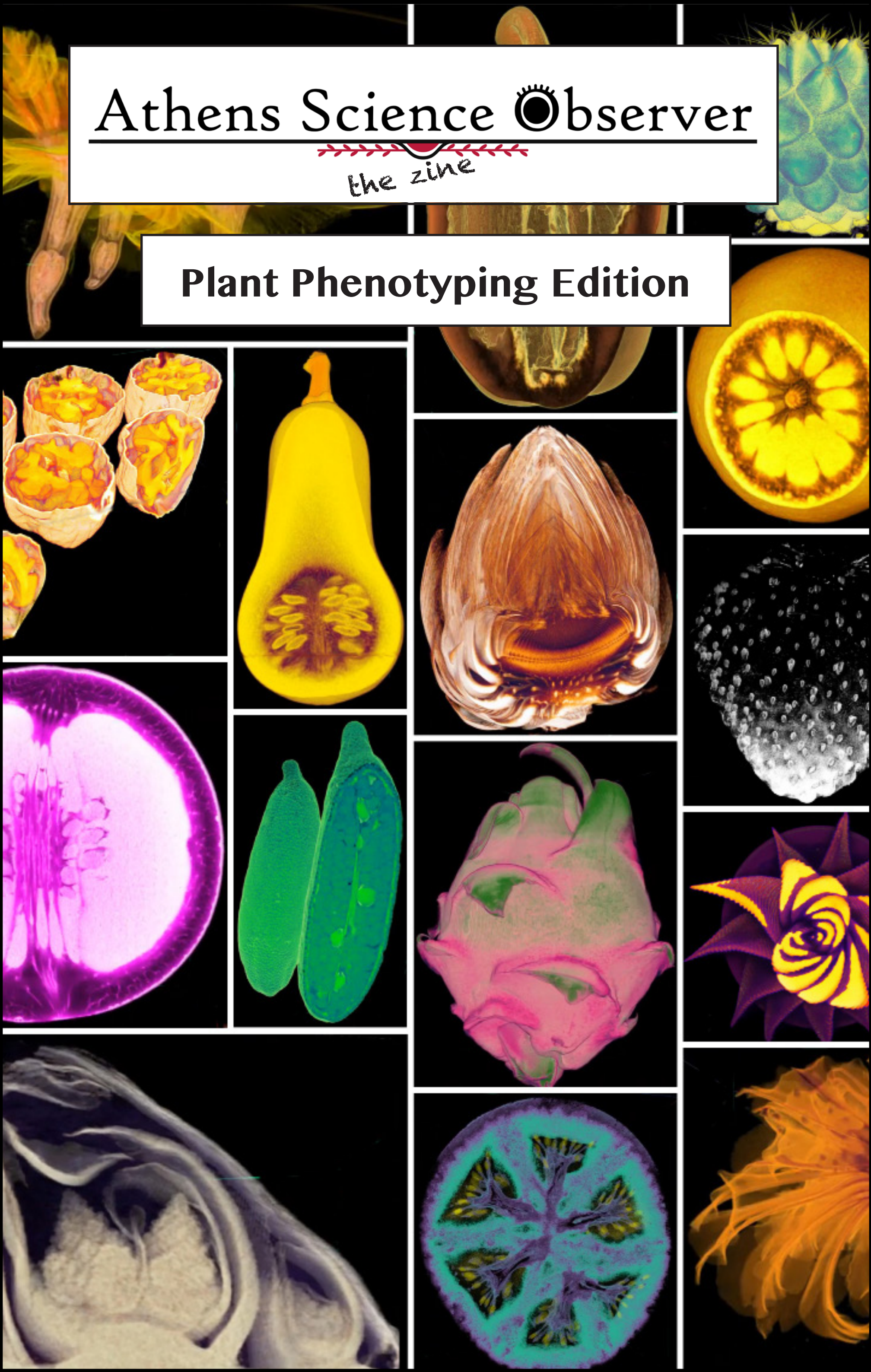
Athens Science Observer

the zine

Plant Phenotyping Edition



Hello!

This Zine is a public companion to the plant phenomics annual science conference being held in Athens Feb. 22 - 25, 2022. At this conference plant phenomic experts from around the world will gather in-person and virtually to discuss the latest impacts, advancements, and generally cool science they've produced over the last year.

But, what is plant phenomics? Textbooks would define this as the measurement of all plant physical and biochemical traits (phenotypes) that can be produced by an organism over the course of development and in response to genetics and environmental influences. Essentially, it's the look, feel, smell plants have naturally, and in response to outside stimuli. There are countless plant phenotypes from the number of seeds in a given sunflower head, to the levels of aromatic compounds produced by plants and herbs, to the specific blade length of a given seaweed frond. But it's not only the phenotype itself these researchers are obsessed with, but also how one measures phenotypes. How do you measure roots under the soil? How do you get usable data from a 10,000 acre corn field quickly and cheaply? How can you remotely measure phenotypes of plants?

With technological advances, the area of plant phenomics is becoming increasingly popular in both basic and applied plant sciences. Through this emerging field we can better improve crop yields; generate controlled environments and methods for vertical farming or growing plants in space; more clearly understand how plants and soils can impact our changing climate. Encompassing food, drinks, pharmaceuticals, fiber, and fuel plants are an integral part of our society, and being able to measure them quickly and accurately will be a large area of scientific study in the next half century.

The conference is sponsored by the North American Plant Phenotyping Network (NAPPN), a 501(c)3 non-profit of plant scientists, engineers, physicists, computer scientists and mathematicians dedicated to plant phenomics. The NAPPN mission is simple: accelerate the visibility and impact of plant phenotyping research being done in North America. This includes funding research, encouraging and highlighting early career scientists, and disseminating work from interdisciplinary fields and diverse individuals to advance the field. Find out more at www.plantphenotyping.org.

-Dr. Rishi Masalia,
conference organizer

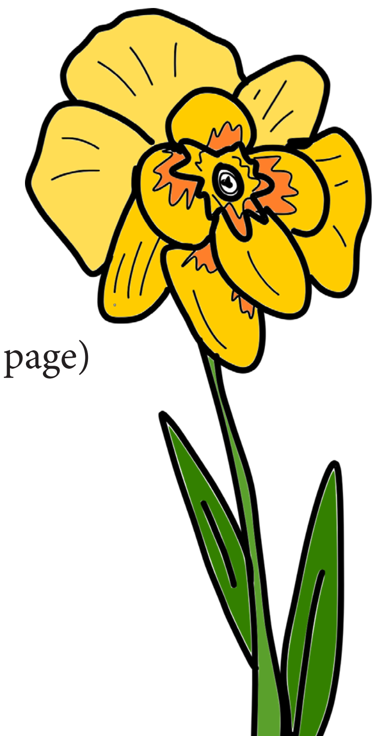
zine

The Zine Team

Editors
Simone Lim-Hing
Dr. Rishi Masalia

Reviewers
Jillian Makala Allen
Max Barnhart
Summer Blanco
Kendall Clay
Kelly McCrum
Jennifer McFaline-Figuera
Audrey Ward

Photo & Art Contributors
Erik Amézquita
Kendall Clay (pgs. current, 1, 2,)
Dr. Rishi Masalia (pgs. 3, 10)
Dr. Suxing Liu (back page)
Dr. Michelle Quigley, Dr. Dan Chitwood, Erik Amézquita (front page)



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Why is everyone in town wearing a lanyard?!

This edition of the ASO Zine is to highlight the annual NAPPN conference, which is being hosted in our very own Athens, GA. Scientific conferences are events for scientists and non-scientists alike to share their work and ideas. These events highlight what's trending, the latest breakthroughs, and current issues in the field. An intended byproduct of scientific conferences is collaboration and communication across communities, institutions, and even disciplines.

With so much socializing and interaction, learning names (and actually remembering them) becomes close to impossible – so dawning the trusty lanyard is almost always the first thing conference goers do after signing in - as if to say, "Hello Conference, I'm here!"

Lanyards are a staple of scientific conferences, since they have your name and often your institution, position, and pronouns.

So if you're around downtown on the week of February 22 and see some folks wearing lanyards, say hey and welcome them to Athens!

Plant phenotying: the future of farming

Ankita Roy, University of Georgia

Simone Lim-Hing
University of Georgia

Crops that are stress-resistant have become invaluable for our food security, especially in light of an increasing population and changes in the climate. Farmers and scientists have been working towards the development of stress-resistant crops since the last few decades, but one bottleneck they face is the accurate measurement of plant traits. Traits are fundamental units of a plant which can be linked to its functions, quantifying either the anatomy, growth, metabolism, or physiology.

Being able to define and describe these traits is pivotal to improving crops - it enables the analysis of plant structure in correlation to functions, like the uptake of water and nutrients by roots or the size and shape of a leaf. Agronomists can then select and breed for these desired traits, i mproving yield, stress tolerance, etc.

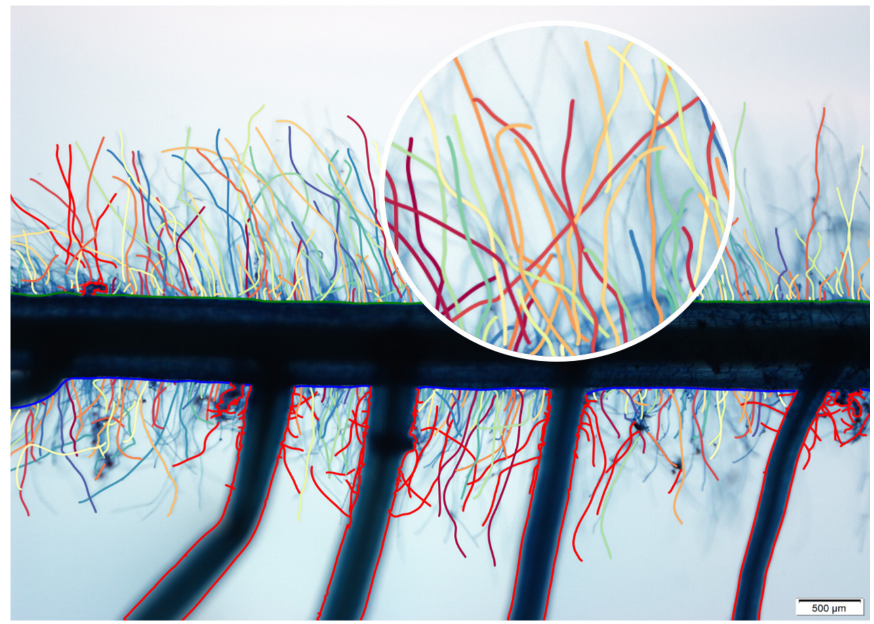
Addressing this bottleneck of the high-throughput quantification of plant traits is the basic concept behind phenotyping. The technical definition of phenotyping is - 'A set of methods for the precise measurement of traits related to the growth, architecture, and genetic composition of plants at all scales of organization ranging from individual cells to the large tree canopies'.

For instance, scientists looking to quantify the canopy size of trees as a function of fruit size. Phenotyping allows the investigators to define characteristics that lend insight into this relationship, which can later help with management and pruning strategies that can improve the crop in the field.

So, how do we implement these ideas? Phenotyping tools can help provide the appropriate answers. Several sensors and computer vision tools have been developed for facilitating high-throughput phenotyping. For instance, canopy size in trees can be calculated from images taken by either satellites or unmanned aerial vehicles like drones. Root architecture can be captured in CT scans and MRI images of the plant taken below-ground. For the correlated functional traits, the rate of water loss can be quantified by a potometer whereas nutrient uptake can be analyzed by spectroscopy.

Tools such as these are a lynchpin in the field, and as we'll see in this zine edition, they are just the tip of the iceberg in the phenotyping world.

Phenotyping research has exploded in recent years, with more than 2,000 scientific publications utilizing this approach have been reported since 2010. The two biggest phenotyping organizations of the world are the EPPN based in Europe (European Plant Phenotyping Network) and NAPPN (North American Plant Phenotyping Network) based in the USA. China has its own network called the CPPN (China Plant Phenotyping Network) in Asia which organized the IPPN symposium (International Plant Phenotyping Network) in 2019 in Nanjing.

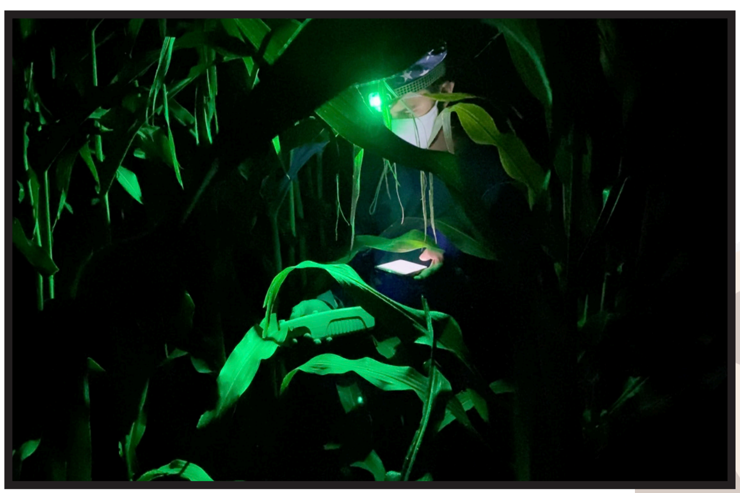


Peter Pietrzyk, University of Georgia
A microscopy image of a maize root with root hairs automatically extracted using our software DIRT/µ. The different colors indicate individual root hairs.

Still, a lot of potential improving crops remains unexplored, especially as the demand for technical expertise in phenotyping keeps rising every day. Embracing technological advances and collaborations between the engineers, biologists, and farmers can allow us to make great strides in this developing field, especially now in this age of climate change.

Day & night...

Dr. James Schnable and his team at the University of Nebraska lead an investigation on the variation in the health of photosystems across 2,000 maize research plots growing at their research farm in Lincoln, Nebraska. Above is photo of Marcin Grzybowski in 2020 using a plant sensor to collect data in the daytime. Below, John Turkus collects the same data at night in 2021.



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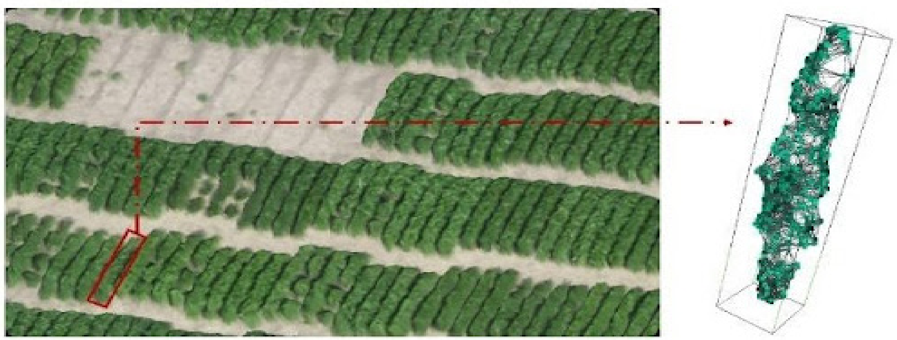
3D modeling applied to plant science

Monica Herrero-Huerta
Purdue University

The planet's steadily growing population faces major challenges including climate change, environmental degradation and food insecurity.

Advances in plant science technology based on sustainable agricultural practices can help ease and even overcome these challenges. Overall, my scientific mission is to develop innovative approaches that may

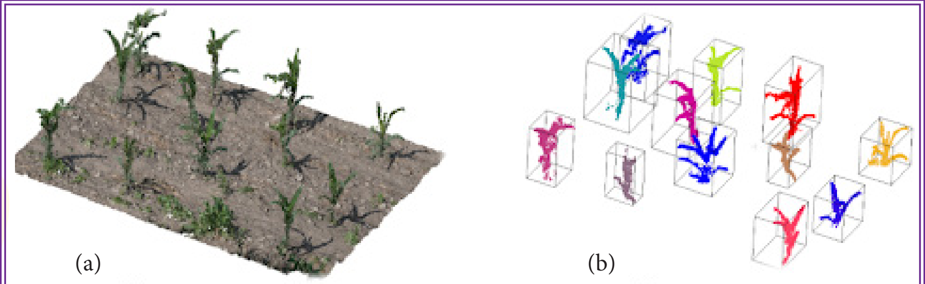


(a) (b)

3D model in soybean (a) and row geometric analysis (b).

Digital innovations for in-field phenotyping

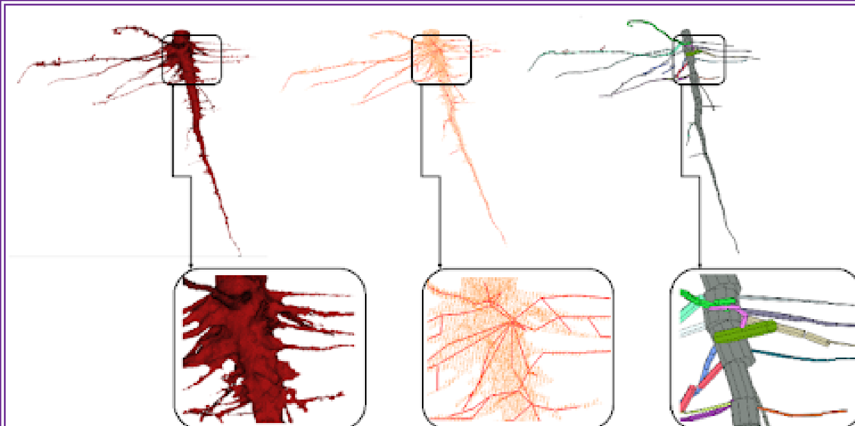
Plant architecture is a 3D feature directly related to crop productivity, fertilizer application and pest control. Unmanned Aerial Systems (UAS) are a highlighted tool for plant phenotyping, which offers the possibility to obtain 3D models constructed from 2D images taken from various viewpoints. I evaluate the potential effect of the 3D shape of plant architectures in-field conditions.



3D model in corn (a) and individual plant extraction (b).

Root modeling by X-Ray Imaging

Roots establish the connection between plants and the soil environment. They are not only the critical piece to water and nutrient extraction, but also assist in carbon sequestration. Deeper rooting crops enhance soil organic carbon sequestration from the atmosphere. Accurate description of root traits helps breeders select for favorable root characteristics regarding crop production, soil degradation and carbon farming. X-Ray technology can non-invasively scan roots. In this context, I developed a spatio-tempo ral root model by first, a skeleton extraction and, second, by a cylindrical fitting. Being able to quantify root traits is the starting point to explore and analyze root system architectures (RSA).



(a) (b) (c)

Tomato root: root scan (a), skeleton (b), and cylindric model (c).

Describing Demeter

Erik J. Amezquita
Michigan State University

Shape plays a fundamental role in plant biology. Shape is tightly linked to behavior, function, and origins of plants and their tissues. Throughout history, from primitive cave paintings to beautifully detailed pictures, the shape of plants has been carefully observed and documented, fueling our biological understanding. As hand drawn figures are replaced by high-resolution scans and vast amounts of drone imagery, the transition of plant biology to a data-driven era has produced extraordinary images from the most diverse organisms, optics, and dimensions.

If we scan organisms with X-rays, we can observe the shape of all internal branching structures and vasculature of roots, shoots, and fruits. These scans are even more revealing when taken as part of a computed tomography (CT), which reconstructs the organism as an actual 3D object. Aided by image processing, outer layers can be easily peeled, and different tissues of interest be separated. With the X-ray lens, for example, the oil glands in the skin of a lemon resemble a constellation of stars. A grapevine bud is a treasure chest ready to sprout. The secrets guarded by thick artichoke leaves are revealed. Every tomato carries a magical forest inside. A pummelo and a supernova explosion are not that different when examined with the right filter. Suddenly, a wonderful universe of shapes opens before our eyes.

Smooth transformations do not alter the topologic shape. A 3D scan of an *Arabidopsis thaliana* leaf subjected to various stretches and smooth deformations.

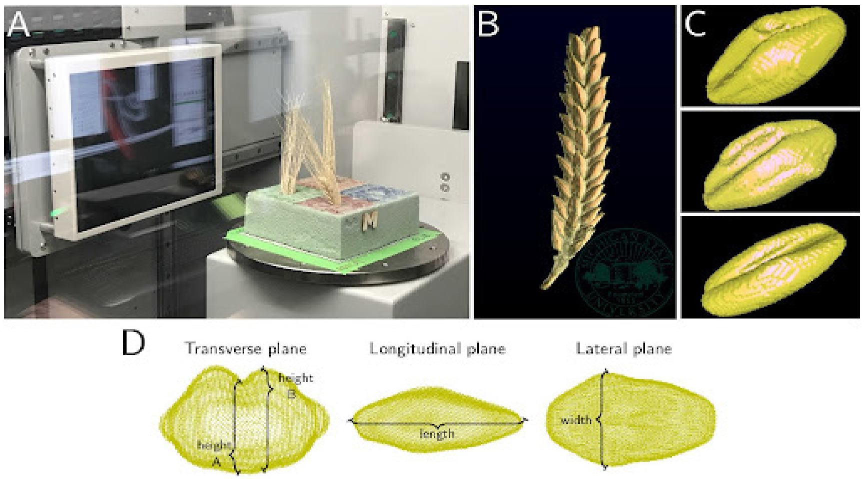
For example, think of the shape of barley seeds. Barley is the 4th most cultivated cereal crop in the world, just behind maize, wheat, and rice, and an ubiquitous source of cattle feed and beer. Barley has helped humankind reach lands far and wide, extending from the warm Mediterranean coasts to the cold Himalayan peaks. Barley's versatility in climate adaptation is accompanied by diversity in its shape and size, both of spikes and seeds. By understanding the relationship between barley traits and its morphology, we can further promote better breeding of cereal crops.

To compare this ever growing shape diversity, we turn to Topological Data Analysis (TDA). TDA is a fast-growing discipline that measures shape comprehensively using mathematical representations based on *algebraic topology*. Topology thinks of the world as if it was made of clay: the shape of an object does not change if we stretch it, shrink it or mold it *smoothly*. Breaking, cutting, and pasting however are not smooth operations, and alter the shape of objects. Topology provides an exact framework to encode changes of shape and to compute *shape signatures* from different objects, like leaves and seeds. TDA allows us to compare diverse grain shapes all at once, free from landmark limitations, for both 2D and 3D images and objects. We have an exact way to say how droopy *is* a droopy leaf and how heart-like *is* a heart-shaped peach, and even describe morphological nuances that are not obvious to the naked eye.

We focus on the shape of barley here at the Morphology Lab at Michigan State University, led by Daniel Chitwood and Elizabeth Munch. We base our first studies on a collection of 28 different accessions or varieties from diverse regions across the Eurasian continent. After scanning almost 1000 spikes (formally known as panicles, the branching inflorescence) we managed to isolate 38,000 individual seeds. In the traditional sense, we can quantify seed shape by measuring their width, length, surface area, or volume. However, looking at the central crease and its subtle texture, we know that there is more morphological information to take into account.

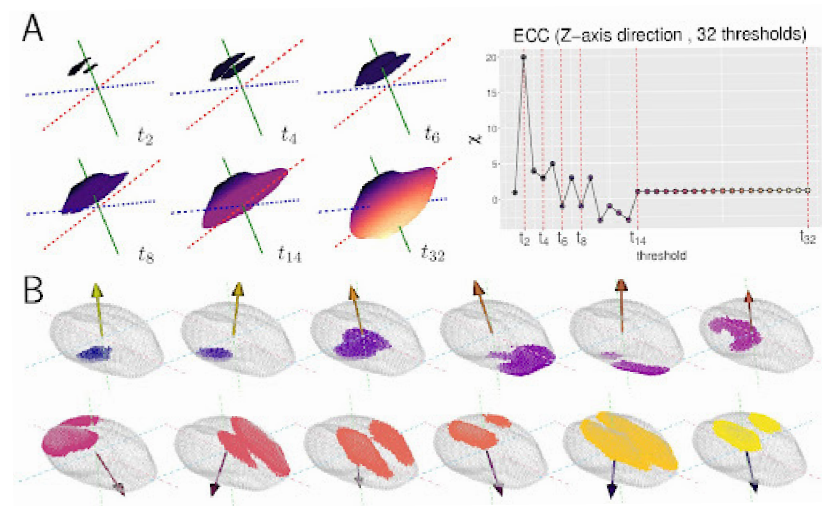
We turn then to topology for a more comprehensive analysis, specifically the Euler Characteristic Transform (ECT), one of the many tools TDA has to offer. The ECT operates as follows. Pick a direction, say top to bottom, and vertically chop the seed into a fixed number of slices of the same thickness each. As mentioned above, slicing and stacking are not smooth operations and it generally alters the topology of an image.

Our goal then is to measure how the Euler Characteristic, a topology-associated number, changes as we stack one slice at a time. If two seeds have similar topological changes as we slice and stack them vertically, then these two seeds must have similar vertical shapes. To take into account the overall shape, we repeat the same slicing and stacking procedure for every direction. Left to right, back and forth, and so on. We can even prove mathematically that if we measure all topological changes across all possible directions, then we have effectively summarized all information related to morphology. This information is sufficient to even reconstruct the original object!



Barley image processing and traditional measurement. (A) 3D X-ray CT scans of the barley panicles. (B) Densities normalized, air and other debris removed, and awns pruned. (C) An extra digital step segmented the individual seeds for each barley spike. (D) The seeds were aligned according to their principal components, which allowed us to measure a number of traditional shape descriptors.

We thus encode seed morphology with two sets of numbers. On one hand we have numbers associated with traditional phenotypes like seed volume and length. On the other hand, we have numbers associated with the inner topological structure of the seed. These numbers may come in patterns, and a computer might be able to separate patterns corresponding to each of the 28 different barley varieties. When using solely traditional shape information, we observed that a computer correctly identifies the variety of the seed 55% of the time. Not a bad result. However, if we also provide the computer with



Topological measurements of the seed shape. (A) We chop the seeds into 32 slices from top to bottom. As we add every slice, we compute a topology-associated number. (B) The most meaningful slices correspond to the seed's crease and bottom morphology.

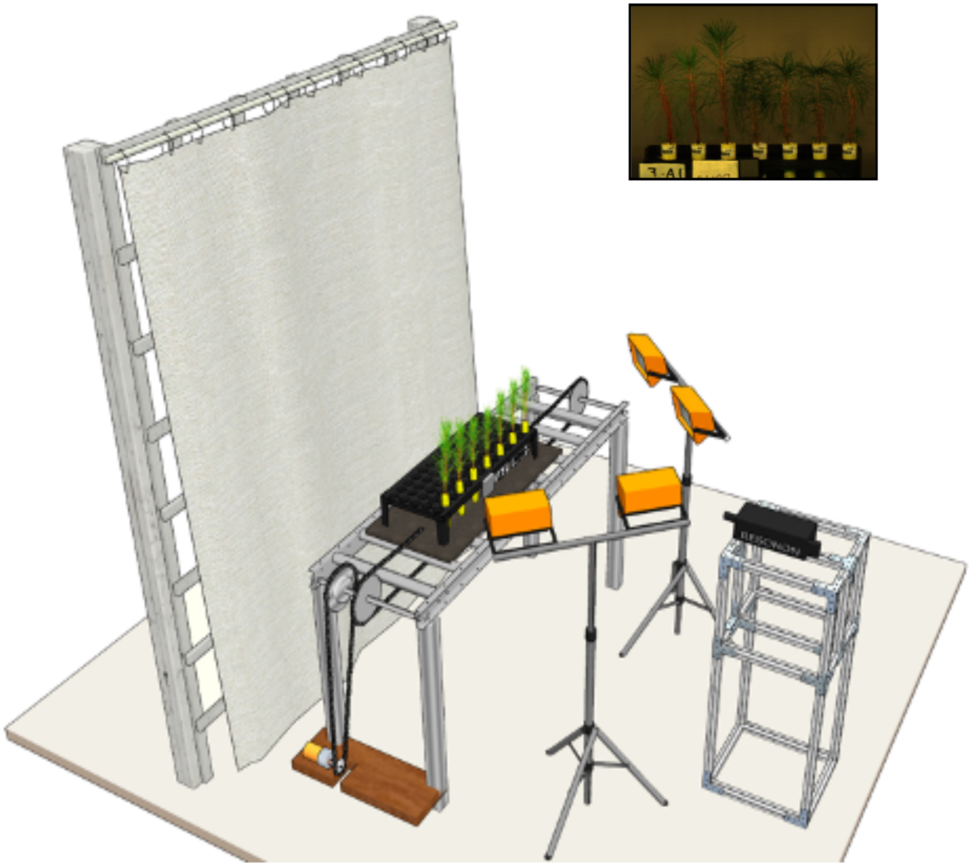
topology-related information, its classification accuracy jumps beyond 85%! Topology actually brings new morphological information to the table.

Next we can analyze what topology is actually measuring. What traits are captured by the topological lens but missed by the traditional ones? We can look for slices that on one hand are the most topologically distinct between different seed accessions, while on the other, these slices remain similar between seeds of the same accession. Formally, this is referred to as a variance analysis. In this case, slices corresponding to the crease and bottom of the seed are quite discerning for different barley varieties.

There is more than meets the eye. Our results suggest that TDA can provide simple yet insightful tools that reveal morphological nuances not captured by geometric morphometric methods. TDA tools can be quite versatile as well; these can be adapted to any kind of images, networks, phylogenetic trees, time series, and many more biology-related objects. We can explore further the phenotype-genotype relationship, and a new, exciting, morphology-driven path is possible. The vision of TDA, that data is shape and shape is data, will be relevant as biology transitions into a data-driven era where meaningful interpretation of large datasets is a limiting factor.

Phenotyping for disease resistance

Piyush Pandey
North Carolina State University



A low cost portable hyperspectral image acquisition system was used with a Resonon Pika XC2 to capture images of loblolly pine seedlings. The seedlings placed on a tray are moved across the field of view-of the line-scanning hyperspectral imager using a motorized chain conveyer. Halogen lamps are used as additional lighting and a gray cloth is seen in the image providing a uniform background. The upper right of the image shows an RGB composite of a typical image captured for multiple seedlings on a tray. Using machine learning methods, diseased and non-diseased seedlings (with fusiform rust disease) were classified based on the hyperspectral data from the stem of the seedlings.

Loblolly pine is the most widely planted tree in the southeast and brings the state of Georgia millions in revenue each year. Fusiform rust is a disease that causes major damage to trees and results in significant losses in yield.
Detecting disease requires thorough visual inspection of each tree - a time consuming and error-prone process.

Piyush Pandey and colleagues at North Carolina State University are using hyperspectral imaging to objectively identify diseased seedlings.

Loblolly pine stem infected with fusiform rust. Photo by Colton Meinecke.

Loblolly pine stands can easily be found throughout the southeast. The industry provides Georgia with tens of thousands of jobs. Photo by Colton Meinecke.

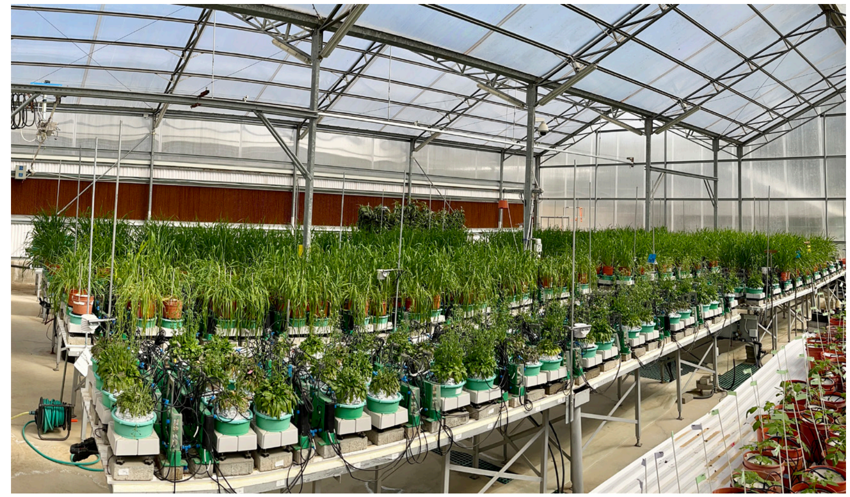
Functional physiological traits phenotyping

Ahan Dalal
The Hebrew University of Jerusalem, Israel

The needs of our increasing global population, the current crop yields need to double by 2050. One major concern is the loss of crops due to adverse environmental factors, such as drought, heat, etc. There are many crops in commercial agriculture designed to tolerate stress from living organisms, such as pests or viruses. However, despite extensive research and patenting, there are not many marketed commercial seeds for crops designed to withstand environmental variation like drought and heat (abiotic stresses). This emphasizes the complexity of the plant response to abiotic stresses. Environmental factor/s (E) being a dynamic factor, its interaction with the genetic features [genotype (G)] is complex, and contributes to the phenotype (observable and measurable characteristics/traits). Some traits are more plastic (less stable; e.g., number of leaves) than the others (canalized or less variable; e.g., shape of leaf).

The complexity of trait plasticity resulting from the dynamic G × E interaction creates a major gap between successful breeding and yield improvement. The bottleneck for the understanding of trait plasticity is the genotype-phenotype gap. To this end, various high-throughput phenotyping (HTP) platforms have made non-destructive phenotyping of large plant populations possible over time. Phenotyping is the assessment of expressed traits. HTP screening techniques and technological aids (e.g., conveyor belts, robotics, gantries) allow the measurement of traits in massive numbers of plants within a relatively short period of time, beyond what would be possible in hand-operated techniques. Most of these HTP platforms assess phenotypic traits through electronic sensors or automated image acquisition (using cameras). Sensor and image data need to be integrated with other multi-omics data (omics refers to the collective characterization of pools of biological molecules/traits) to create a holistic, second-generation phenomic approach.

Apart from challenges in translating sensor information/readings into knowledge, the reliability and accuracy of currently available imaging techniques for in depth phenotyping are questionable. Most use controlled/defined conditions (very different from a true fluctuating environment), thus the experimental results from controlled environments are difficult to translate to that from the field. Finally, the entry price of image-based HTP systems is very high, which also requires higher standards of growth-facility infrastructure and significant maintenance.



The Plantarray System

Yield-related physiological traits [e.g., photosynthesis, transpiration, etc.] typically depend on the cumulative actions of many genes and the environment and are known as quantitative traits. These quantitative physiological traits (QPTs) can be ranked based on their degree of plasticity and grouped from lower (e.g., molecules, cells, tissues, etc.) to higher levels (e.g., organs and organisms). More plasticity is seen at the tissue and cellular levels which contributes to the stability of the QPTs found at the organism level, thus helping in survivability. While breeding programs have improved productivity by canalizing traits that are responsible for increased water use, they have also increased the plant susceptibility to environmental stressors. A better understanding of the mechanism controlling QPT stability would enable us to develop

In the laboratory of Prof. Menachem Moshelion at Hebrew University in Israel, we developed an HTP-telemetric phenotyping platform designed to solve many of the problems mentioned above. Telemetry technology enables the automatic measurement and transmission of data from remote source(s) to a receiving station for recording and analysis. Our non-destructive HTP-telemetric platform includes multiple weighing lysimeters (scales) and environmental sensors. This system can be used for the collection and immediate calculation (image-analysis is not needed) of a wide range of data, such as whole-plant weight gain, transpiration rates, CO2/O2 exchange, root growth, etc.

This has great value for practical decision-making, a substantial improvement from what can be acquired from controlled environment phenotyping experiments and greenhouse studies of drought stress. Other advantages of the telemetry platform are its scalability, ease of installation, and its minimal growth-facility infrastructure requirements. Moreover, as this sensor-based system has no moving parts, both the entry price and long-term maintenance costs are relatively low.

Unlike image-based HTP platforms, whose measurements are limited to morphological and indirect physiological traits, our whole-plant physiological HTP-telemetric platform takes continuous, high-resolution, direct physiological measurements, and can accurately detect the physiological stress point of each plant. This enables the researcher to monitor the plants, make decisions regarding how the experiment is to be conducted, and determine how many samples are to be collected over the course of the experiment. This system takes measurements at field-like plant densities, which eliminates the need for either large spaces between the plants or moving the plants for image-based phenotyping.

This HTP-telemetric physiological phenotyping method might be helpful for conducting greenhouse experiments with close-to-field conditions. This system's abilities are extremely important in the pre-field phenotyping stage, as they offer the possibility to predict yield penalties during the early stages of plant growth. The real-time analysis using our HTP- telemetric platform, represents an important step in the translation of numbers into knowledge, thus might contribute to an important step towards food security and global demands.

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Glossary of Terms

Abiotic stress	The negative impact of non-living (biotic) factors on living organisms in a specific environment. E.g., drought, salinity, low or high temperatures.
Computer vision	An interdiciplinary field that deals with how computers can gain high-level undestanding from digital images or videos. Sub-domains include object detection, object tracking, and image restoration
Genotype	The genetic makeup of an individual
High-throughput plant phenotyping	Technologies that offer the ability to non-destructively image a large number of plants over time, for specific traits.
Machine Learning	The study of computer algorithms that can improve automatically through experience and by the use of data
Morphological Trait	Changes to the outward appearence, form, structure
Phenome	The measurement of all physical and biochemical traits (phenotypes) that can be produced by an organism over the course of development and in response to genetics and environmental influences
Phenotype	A set of observable traits of an organism. E.g., flower color, berry sweetness
Plant Biomass	Weight of living material. This can be separated by different plant organs (roots, leaves, etc)
Plasticity	The ability for a given trait to change to better cope with its environment
Root architecture	A collection of root-specific traits that determine the spatial and temporal distribution of roots in the soil (or other growth matrix), and thus the ability of plant roots to obtain resources.
Topology	The study of geometric properties and spatial relations unaffected by the continuous change of shape or size of figures; the way in which constituent parts are interrelated or arranged.
Vasculature system	In plants, the vascular system is the assembladge of conducting tissues and support fibers that transport nutrients and fluids throughout the body. In humans this is the circulatory system.

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