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Anatomics: High-throughput phenotyping of plant anatomy

Christopher F. Strock,[1,4] Hannah M. Schneider,[2,4] and Jonathan P. Lynch[3,*]

Anatomics **is a novel phenotyping strategy focused on high-throughput imaging and quantification of plant anatomy from field-grown plants. Here we highlight its potential applications for genetic and physiological analysis of plant anatomical phenotypes.**

The challenges of phenotyping plant anatomy

A fundamental feature of plant form and function is the structure and organization of cells and tissues. Plant anatomy is a key regulator of fundamental processes, including photosynthesis, the acquisition and transport of water and nutrients, metabolic costs of constructing and maintaining tissue, tissue biomechanics, and interactions with other organisms (Figure 1) [1].

Measuring and analyzing anatomical phenotypes have been research bottlenecks, resulting in gaps in our understanding of the extent of phenotypic variation among taxa and how this variation relates to fitness. Methods for high-throughput anatomical phenotyping would benefit many domains of plant science, ranging from basic research to crop breeding. For example, methodological challenges are a primary reason why anatomical phenotypes are underused in crop breeding programs, despite being attractive targets for the development of more efficient, resilient crops [1].

Anatomical phenotyping has been limited by throughput as well as potential bias in the source of plant material commonly visualized with existing methods. Many studies of plant anatomy use tissue derived from young plants from artificial environments, although such phenotypes may be poor predictors of mature phenotypes in the field. Here we outline a phenotyping pipeline termed *Anatomics* that prioritizes high-throughput imaging and quantification of plant anatomy from mature, field-grown plants. We discuss methods for image capture and analysis that contribute to the field-focused, high-throughput objective of *Anatomics* and advocate for implementing this approach for the genetic and physiological analysis of plant anatomical phenotypes.

Imaging plant anatomy

Light microscopy is well suited for imaging superficial structures such as root hairs, trichomes, and stomata. Inexpensive portable microscopes permit rapid, in-field imaging where access to benchtop microscopes is impractical [2]. Continued advancements in cost, compactness, and performance of portable microscopes will make them an integral tool for high-throughput, field-based imaging in *Anatomics* across multiple disciplines, including ecology, physiology, and crop breeding.

For imaging internal structures, the advent of laser ablation tomography (LAT) addresses gaps in sample throughput and spatial scale that are unfulfilled by existing methods [3,4]. LAT represents a breakthrough for image capture in *Anatomics*. LAT uses a pulsed UV laser to ablate a sample while a high-resolution camera images the exposed surface, producing full-color images at spatial scales from 0.1 mm to 1 cm with micrometer-level resolution (Figure 2). Using LAT, tissue does not require time-consuming preparation, and imaging takes between 1 and 2 minutes per sample. Similarly, a vibrating microtome can be employed for rapid sectioning to increase the throughput of image capture with traditional microscopy [5].

Although 2D image capture is a foundational objective of *Anatomics*, 3D imaging is important for understanding processes such as gas diffusion, light absorption, water transport, and biomechanics [6]. Techniques such as confocal microscopy, NMR, micro–computed tomography (micro-CT), and LAT all have unique capabilities and limitations in their capacity to capture anatomy in 3D. Although NMR and micro-CT are nondestructive [6], the limited throughput of these methods diminishes their utility for *Anatomics*. Confocal microscopy is well suited for 3D imaging but is low throughput and is constrained by tissue opacity. Although LAT can rapidly generate 3D datasets and the depth of a sample scanned is not limited by tissue opacity, it is destructive [4,7].

In addition to imaging structure, *Anatomics* seeks to quantify tissue composition. Microscopy augmented by histochemical staining and fluorescent reporters have existed for decades, but the recent development of label-free imaging technologies opens new opportunities for understanding chemical composition of plant tissue [8]. Similarly, LAT serves as a label-free method capable of revealing cell wall composition via UV fluorescence. Although differences in autofluorescence of different tissues are measurable across the spectrum of visible light using an RGB (red, green, blue) camera, further development of LAT to accommodate hyper- or multispectral imaging has even greater potential to detect tissue composition.

Image analysis and data extraction

Once images are captured, accurate quantification of biologically meaningful phenotypes is another challenge. Phenotypes of interest may be defined by the dimensions, abundance, distribution, or spectral qualities of anatomical features.

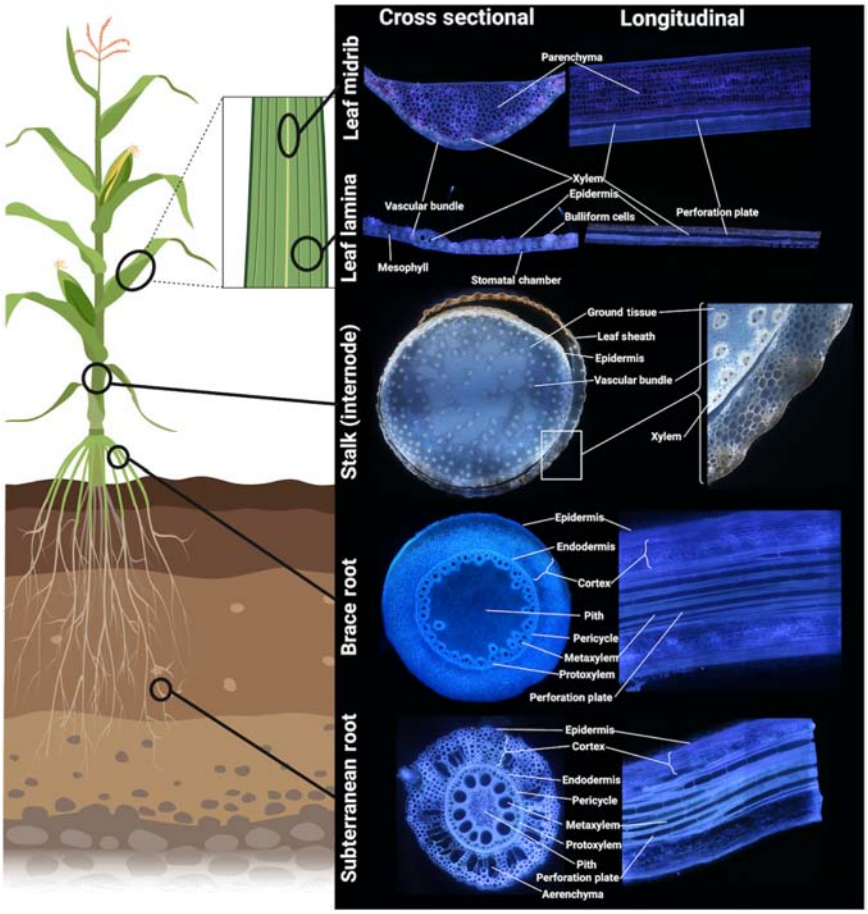

Figure 1. Examples of anatomical features that can be visualized throughout various plant organs using laser ablation tomography. Here we show cross-sectional and longitudinal views of leaves, brace roots, and subterranean roots in field-grown maize plants. This figure was created using BioRender (https://biorender.com/).

Because a core objective of *Anatomics* is to determine the genetic control of anatomical phenotypes, accurate and precise measurement is crucial for the identification of genes that may have small allelic effect sizes.

Extracting quantitative data from anatomical images requires the resolution and segmentation of specific tissues or cells. Depending on image complexity and consistency, feature identification and measurement may employ manual, semiautomated, or fully automated processes. Although generic software, such as ImageJ, offers user-friendly tools as well as adaptability and automation through macro-directed batch analyses, such tools are not designed for rapid processing of more complex features across diverse tissues and taxa. Nevertheless, a variety of open-source and commercial platforms that rely on traditional preprocessing and segmentation techniques are available to develop fully automated image analysis pipelines for specific applications [9]. Automation using traditional segmentation methods works well where sample collection and image capture methods are uniform and features are consistent in size, shape, contrast, and position. Extensive modifications of image processing are required when applied to diverse image sets, and pipelines using traditional methods often benefit from the inclusion of semiautomated processes that require user input. More sophisticated detection methods such as template matching, maximum stable external region extraction, and wavelet spot detection have been used to automate the identification of features to good effect [10].

Advances in computer vision and machine-learning techniques will be useful for improving the throughput of image analysis in *Anatomics*. Deep learning approaches that build on convolutional neural networks have already been employed in a variety of plant phenotyping tasks [11]. Nevertheless, such methods often require a large amount of training data and currently excel at extracting data from a specific sample type and collection procedure [10].

Increasing capacity in computing power and image analysis tools will be especially useful for the quantification of 3D structures. 3D analysis of anatomical features is essential for accurate estimates of volume and surface area, understanding spatial relationships, and analyzing flux networks [6]. This is a nascent area of research limited by computationally demanding image processing methods that require extensive human interaction.

For a full array of image analysis software for *Anatomics*, a repository of open-source tools can be found at https://www.quantitative-plant.org [9].

Case studies of *Anatomics*

In *Anatomics*, throughput of image capture and analysis from field-grown samples is essential to phenotype large populations and conduct genetic analysis. The utility of *Anatomics* in this context has been demonstrated in several recent studies where it was used to

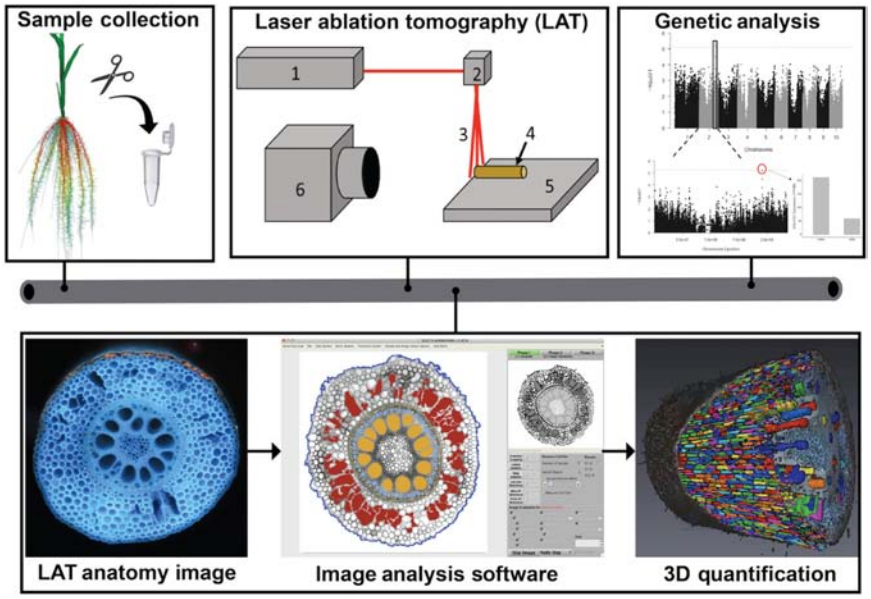

Figure 2. Visualization of the *Anatomics* pipeline from sample collection through identification of genetic markers. Samples are (1) collected from field-grown plants and preserved in 75% (vol/vol) ethanol, (2) imaged directly from storage in ethanol with laser ablation tomography (LAT), (3) images are phenotyped using image analysis software, and (4) genetic markers are identified. The LAT is composed of a pulsed UV laser (1), which is modified through beam-shaping optics (2) to create a 'cutting sheet' (3) and directed onto a sample of plant tissue (4). The sample is advanced into the ablation plane by a motorized stage (5) while a high-resolution camera (6) images the anatomy that is exposed as the tissue is ablated.

quantify heritable anatomical variation in field-grown plants. *Anatomics* has been used to phenotype diverse tissues, including roots, leaves, stems, and seeds, as well as across diverse species ranging from monocots and dicots, annual and perennial, landraces and modern cultivars, and tropical and temperate species (e.g., [1,4,7,12,13]). In one example, LAT was used to image approximately 10 000 field-grown maize roots. These images were then phenotyped semiautomatically with *RootScan* software to extract eight anatomical phenotypes related to the root cortex, metaxylem, and stele. A genome-wide association study (GWAS) was then run to identify over 120 candidate genes associated with these anatomical phenotypes and their plastic responses to drought [13].

In another example, *Anatomics* was used to discover a novel anatomical phenotype in cereals, multiseriate cortical sclerenchyma (MCS), which is important for the penetration of roots into compacted soils. LAT was uniquely capable of imaging thousands of maize and wheat root samples to quantify genotypic differences for MCS based on autofluorescent spectra and cortical anatomy. More than 3000 images were phenotyped using *MIPAR* software, and resulting data were used in GWAS and quantitative trait locus mapping to identify and confirm a candidate gene associated with MCS in maize on chromosome 9 [12].

Finally, *Anatomics* may also benefit from increasingly powerful *in silico* tools, by which anatomical phenotypes are used to parameterize functional-structural models that can simulate how variation in plant anatomy may influence processes such as water transport, nutrient acquisition, tissue maintenance cost, and gas exchange [1,14]. Characterizing anatomical variation and using this information to model complex, nonlinear relationships at the anatomical scale will be key in understanding how such variation affects plant and ecosystem function [14].

Concluding remarks

Prioritizing throughput of anatomical imaging and analysis affords researchers the opportunity to characterize the extent of phenotypic variation for plant anatomy, identify the genetic architecture of anatomical phenotypes, and understand the utility of this variation for plant fitness. Technologies that facilitate these research objectives in mature, field-grown plants are fundamental to *Anatomics*. Advances in computing power, machine learning, 3D imaging, hyperspectral imaging, and functional-structural modeling will continue to improve the throughput and breadth of information captured by this pipeline in the future. *Anatomics* will permit greater understanding of plant anatomy in basic plant biology and will have multiple applications in agriculture and ecology.

Declaration of interests

The authors have no interests to declare.

[1]Breeding Insight, Cornell University, Ithaca, NY, USA
[2]Centre for Crop Systems Analysis, Wageningen University & Research, Wageningen, The Netherlands
[3]Department of Plant Sciences, The Pennsylvania State University, State College, PA, USA
[4]Co–first authors

*Correspondence:
jpl4@psu.edu (J.P. Lynch).

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