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Image analysis is driving a renaissance in growth measurement

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The domain of machine vision, in which digital images are acquired automatically in a highly structured environment for the purpose of computationally measuring features in the scene, is applicable to the measurement of plant growth. This article reviews the quickly growing collection of reports in which digital image-processing has been used to measure plant growth, with emphasis on the methodology and adaptations required for high-throughput studies of populations.

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Introduction

Automated methods for measuring plant growth were in use by the end of the 19th century (Figure 1) but even then pioneers like Wilhelm Pfeffer (1845–1920) recognized the potential of early imaging techniques, 'Photographic registration will probably be largely employed in the future, for series of pictures may be obtained which when placed in a kinematograph show the phases of several days' or weeks' growth in a minute or so' [1]. In subsequent decades, researchers devised various photographic methods for studying growth. Computers were eventually brought to the task by digitizing video footage [2] or projecting photographic transparencies onto digitizing tablets [3,4]. As *Arabidopsis* with its great genetic advantages replaced traditional (and much larger) subjects such as oat coleoptiles, cucumber hypocotyls, and pea epicotyls, a millimeter ruler frequently could provide the resolution needed to answer the important questions at hand, such as whether the hypocotyl or root was longer or shorter than the wild type. Lack of need for high resolution coupled with the difficulty of achieving it with tiny *Arabidopsis* seedlings pushed the topic of growth measurement into something equivalent to the Dark Ages. Fortunately, the renaissance is well underway due to the advent of digital image acquisition and computational processing. The combination of high resolution, accuracy, and throughput achievable with today's sensors and computational technologies is allowing growth measurements to be compatible with large-scale, systems-style biology research.

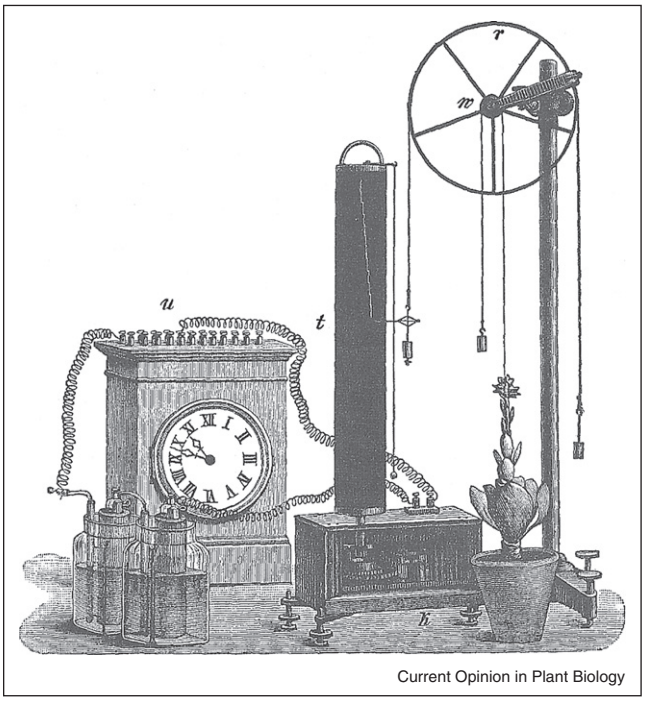
Basic image analysis

Nearly all machine vision solutions applicable to measuring plant growth from images depend fundamentally on segmentation and analysis of structure, two procedural stages that share a blurry border. Segmentation determines the boundaries of human recognizable components of the image that include the objects of interest. Structure analysis is concerned with characterizing curves, boundaries, pixel intensities and their differentials. Early computer vision practitioners recognized and addressed these general issues by devising algorithmic solutions to the challenges of finding lines, corners, and boundaries in digital images [5–11]. Such works continue to serve as the foundation for the image-analysis approaches to plant growth reviewed here. Figure 2a illustrates how segmentation and structure analysis can be combined to measure growth of an arbitrary structure shown at two time points and deliberately blended into the background. Segmenting the object of interest from the background can be achieved with algorithms ranging from those that detect the optimally discriminating threshold of pixel intensity based on the structure of frequency histograms [11] to those which assign each pixel a probability of belonging to an object based on Bayesian statistics [12], to those that utilize machine learning techniques such as support vector machines or neural networks [13–15]. Whatever method is used, the result is a set of object pixels from which the defining contour or boundary (black line in Figure 2b) can be determined. The boundary is used explicitly or implicitly to determine the midline of elongated objects (red lines in Figure 2b) such as seedling stems and roots. Each of the various midline-finding techniques which one can use depends on some determination of the point that lies equidistant between two opposite boundary positions.

Morphometrics

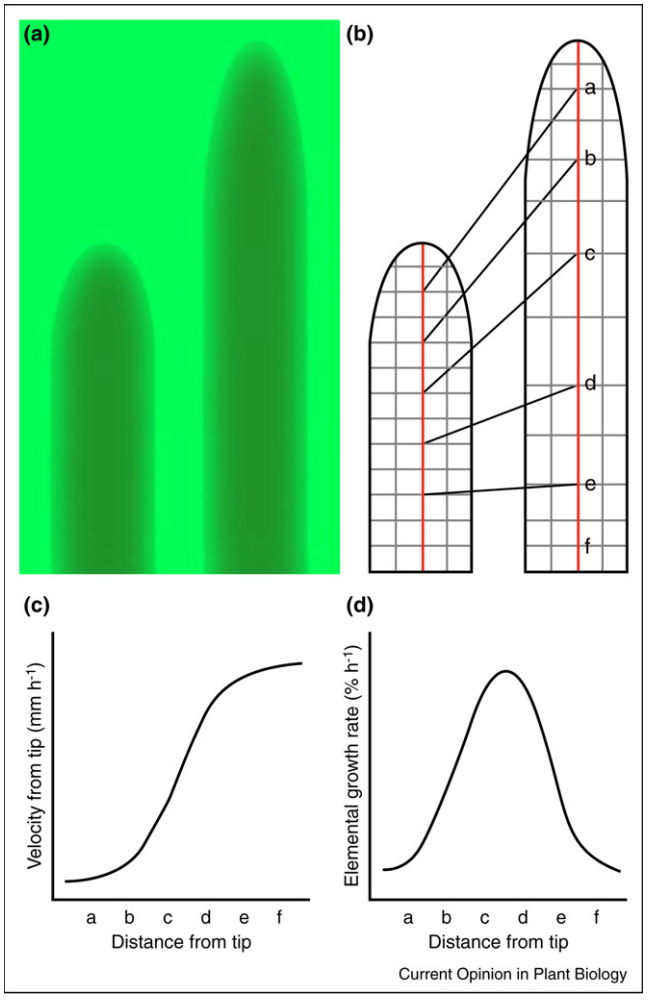
Midline length and the distribution of local curvature along it can give a very useful description of a biological structure such as a plant root or stem [16]. From a time series of images, the rate of change of these morphometric parameters can quantify growth and shape changes with resolution on the order of minutes and microns [17•,18]. An important step in a midline-based growth measurement is detection of the correct termination point. One published solution for tracking growth of etiolated seedlings responding to light used a gap that is usually present

Figure 1



An auxanometer is a device for making automated measurements of growth. A figure of a late 19th century auxanometer taken from Wilhelm Pfeffer's classic textbook is shown [1].

Figure 2



Schematic illustration of how morphometric or kinematic descriptions of growth are obtained from images. **(a)** An arbitrary shape having grown in length during a time step is deliberately made similar to the background to emphasize the fact that its separation from the background may not be trivial. **(b)** Successful segmentation defines the object's boundary (black outline) which aids in the determination of the midline (red line). The gray grid represents fiduciary marks, applied or endogenous, that if matched between images can allow a kinematic analysis of the behavior of the material comprising the object. **(c)** Velocity profile is obtained by determining how fast marks at each of the indicated positions moved away from the tip. **(d)** Elemental growth rate as a function of position is obtained by differentiating the curve in c.

at the base of the closed cotyledons as an identifiable point where the hypocotyl midline is terminated [19]. A technique that worked well for de-etiolated seedlings with opened cotyledons took advantage of a thickening of the hypocotyl at the cotyledonary node [20•]. A third technique that successfully quantified hypocotyl growth responses to ethylene used a local pattern-matching method to terminate the midline at a reproducible cotyledon location [21•]. These methods were either automatic or semiautomatic, which is necessary if the method is to replace standard manual methods and enable population genetic and systems-style studies. In the case of roots, which the object in Figure 2 reasonably well exemplifies, linear extrapolation of an apical subset of midline points intercepts the boundary at a point that has proven useful for termination [18]. The RootTrace tool terminates the midline at the tip by finding the last pixel in a progression having a sufficiently high posterior probability of belonging to the root object [22••].

Kinematics

Whereas morphometrics is the study of geometric features, kinematics is the study of the internal material processes that create the geometry, namely cell production and expansion [23,24]. Kinematic analyses have shown plant growth to be a form of material flow, which has been tracked from sites of cell production by photographing growing organs marked with exogenous [3,4,25,26], or endogenous surface marks [27]. Figure 2b supposes a grid of features to be tracked within the object boundary to illustrate a kinematic analysis of growth. To an observer at the tip of the structure, point 'a' appears stationary over time because cells in that region are not expanding much. A point at location 'b' would move away

from the observer at a slow rate, whereas a point at 'c' would appear to move away considerably faster. Point 'f' moves away from the observer at the maximum rate not because 'f' marks a region of fast material expansion but because the interval includes all of the expanding material. Figure 2c plots the velocity profile just described. The maximum velocity is equal to the growth rate a midline-based morphometric method would measure. Velocity profiles can be obtained by applying optical flow analysis methods to time series of high-resolution digital images. Instead of ink dots, many small patches of endogenous texture in an image caused by refraction of light from cell walls or other optical effects of the tissue can be matched from one frame to the next in a time series [28–31]. Differentiating the velocity profile with respect to position, the *x*-axis, produces the elemental growth rate profile shown in Figure 2d. It provides a kinematics-based definition of the elongation zone and some fundamental information about growth of the primary plant body. For example, *Arabidopsis* is not a small plant because it has a low capacity for growth. The peak elemental growth rate of its root, when measured as just described from images, is 40–50% $hour^{-1}$ [28–31], perfectly matches values obtained for the much larger maize [25,32] and bean [33] roots. Kinematics shows that maize and bean roots are bigger than *Arabidopsis* roots because they have more and bigger cells, and not because each element of material has a higher intrinsic capacity for expansion.

2D versus 3D

The above treatment covered only the analysis of simple structures in 2D images. More complicated images may require more complicated algorithms but not new principles. For example, a branching root system can be approached by segmentation, contour, and midline analysis to produce a skeleton [34,35]. Likewise, adding the third spatial dimension complicates the task but the image analysis steps are some form of segmentation and structure analysis. Perhaps the larger differences between 2D and 3D studies lie in the image acquisition technologies.

Root system architecture in 3D has been studied with diverse imaging modalities. A successful method using visible light depends on acquiring digital images of a root system grown in a transparent medium as the subject is rotated. From the resulting angle series, a back-projection method enables faithful reconstruction of the 3D architecture [36•,37••]. Repeating the acquisition at different time points enables growth studies, one sample per apparatus. X-rays [38], and magnetic resonance methods [39,40] have also been used to obtain 3D reconstructions of root systems in soil, but not of their growth. At the cellular scale, 3D reconstructions of optical slices obtained by laser scanning confocal imaging are commonplace, though obtaining time series from which growth can be measured is far from simple [41••]. Methodologies that measure the path length of reflected laser light may prove to be an effective way to measure 3D growth of plant structures [42,43].

Throughput

Automation of image analysis can allow experiments to expand beyond what would be feasible in a manual-analysis scenario, shifting the rate-limiting step to image acquisition. Throughput of image acquisition can be increased by employing multiple image-acquisition devices, each focused on a separate sample [44]. Another approach is to control the movement of a single acquisition device to parallelize the measurement of multiple samples [21•]. Each approach has limitations or technical challenges to overcome. Setting up parallel experiments in front of multiple devices can be time consuming and difficult to synchronously initiate. Moving a camera to inspect multiple samples may require technically demanding servoing with precision motion-control hardware and software [29,45•,46•]. A third approach is to increase the size of the scene so that multiple samples can be included in a single capture event. Standard digital cameras can capture overhead images containing several *Arabidopsis* plants, for example. Because of the relatively flat profile of the green rosette against a dark soil background, the segmentation step is fairly straightforward. The resolution achieved with such cameras is sufficient to resolve small increments of growth. This scenario has been successful [47,48] and commercial platforms for systematizing the measurements are available (www.lemnatec.com). A more complicated wide-scene image is also a popular data type in *Arabidopsis* research. A standard flatbed document scanner can capture images of multiple Petri plates in one scan, with each plate containing multiple seedlings growing along its vertically oriented agar surface so that potentially large numbers of roots, hypocotyls, cotyledons, and possibly leaves and lateral roots are represented in profile. Typically, the researcher measures the structures of interest using a manual point selection device. Needed to make the inexpensive and easily automated flatbed scanner into a high resolution, high throughput, growth-measuring device are algorithms capable of matching the human's ability to discern and measure the specific structures of interest. Incorporating supervised machine-learning algorithms into the image analysis tool holds much promise in this regard. One hundred years ago, Pfeffer saw image analysis as a way to study plant growth in the future. From here, the perspective seems to be different only in the degree to which throughput, resolution, and precision will increase.

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