

Simple Imaging Techniques for Plant Growth Assessment¹

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Introduction

Quantification of plant phenotypic traits, such as plant height, plant width, stem diameter, and leaf area, is often performed manually in the field or after destructive plant sampling. Such data collection methods are laborious and time-consuming, often requiring multiple people.

There are simple imaging techniques that can perform these measurements more quickly and precisely (Patrignani and Ochsner 2015, Sozzani et al. 2014, Stewart et al. 2007). The basic procedure involves taking a photograph with a digital camera or smartphone and then calibrating and processing the image using an image processing program, such as ImageJ (free to download and use). Figure 1 shows examples of canopy images processed by ImageJ to calculate canopy cover. The advantages of image-based measurements are as follows:

- Time spent in the field is minimal; image processing and measurements can be performed indoors at a convenient time.
- The entire process can be done by one person.
- Image-based measurements are quicker and can be more precise than manual measurements.
- Cost and tools needed are minimal—you only need a compact digital camera or smartphone and ImageJ.
- Multiple measurements can be performed on the same image (e.g., plant width, canopy cover, and fruit counts).
- Photographs themselves are very good visual data.

- Photographs can be saved with metadata, such as geolocation, date, and time, automatically.
- Photographs and measurement data can be archived electronically.

Image-based measurements are useful not only for researchers but also for Extension agents, especially when collecting data from on-farm trials with limited time and resources. The techniques described in this article work well particularly for small herbaceous plants. This article is intended to help researchers and Extension agents collect plant growth assessment data more efficiently using simple imaging techniques.

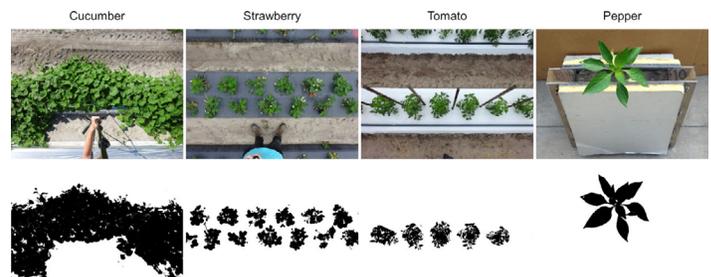


Figure 1. Overhead canopy images of various crops converted to binary images using ImageJ for canopy cover measurements. Credits: Shinsuke Agehara, UF/IFAS

ImageJ

ImageJ is a public domain, Java-based image processing program developed at the National Institutes of Health and the Laboratory for Optical and Computational Instrumentation. ImageJ was designed with an open architecture,

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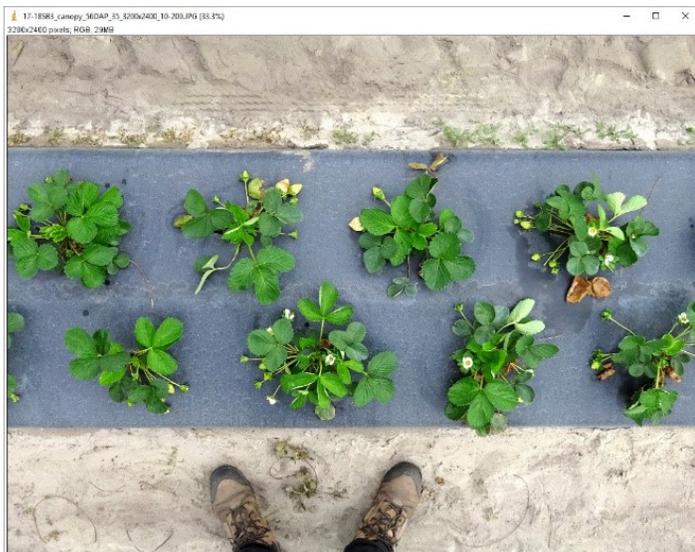
so users can write custom plugins to solve many image processing and analysis problems. There are already many plugins available that allow more advanced measurements, such as three-dimensional live-cell imaging, radiological image processing, multiple imaging system data comparisons, and automated hematology systems.

Download ImageJ Software

1. Go to <https://imagej.nih.gov/ij/> and select “Download.”
2. Determine your platform (Mac/Linux/Windows) and click on the appropriate link.
3. Install the program. The measurements described below do not require plugins.

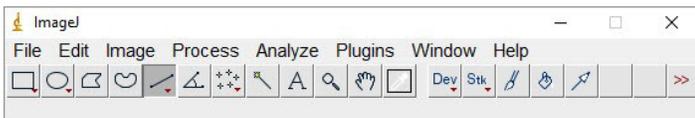
Import Your Image

1. Go to “File > Open” and navigate to your image, or drag the file onto the ImageJ toolbar. Below is an overhead photograph of strawberry plants imported in ImageJ.

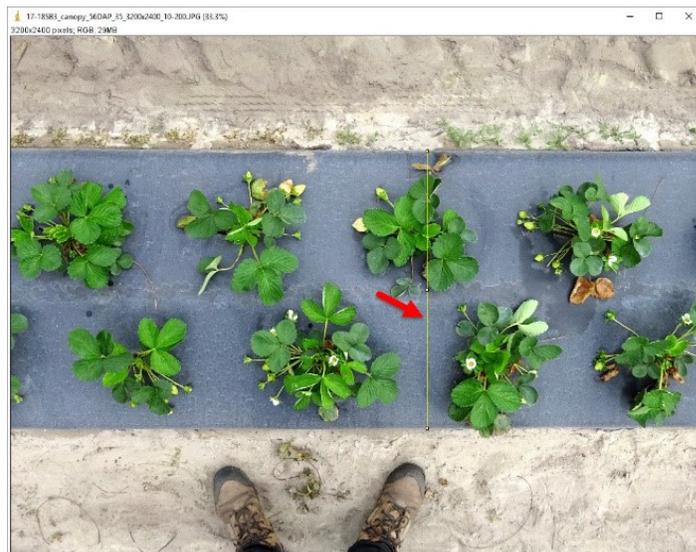


Set Scale (Image Size Calibration)

1. Select the “Straight Line” tool (highlighted below).

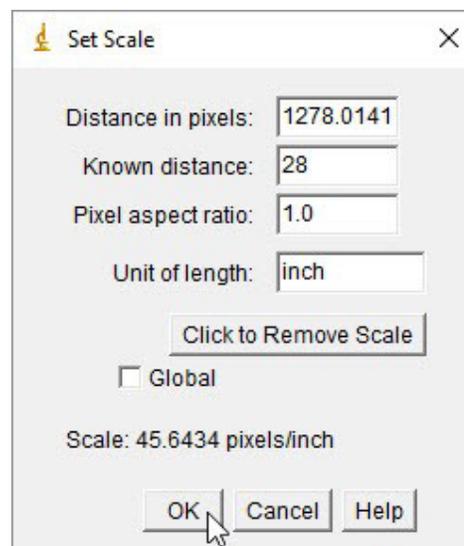


2. Draw a line to an object of known size (e.g., strawberry bed).



3. Go to “Analyze > Set Scale” to set the distance to the line.

- Enter the following parameters (e.g., strawberry bed width = 28 inches).
 - Known distance: 28
 - Unit of length: inch



- Click on “OK.”

Notes:

- For accurate image size calibration, the reference object should be large enough and placed near the object of interest.
- If you have multiple images taken with the exact same distance between your camera and the reference object, check “Global” option, and the same image calibration will be applied to images that will be imported subsequently. Otherwise, “Set Scale” must be done for each image before proceeding to the next step.

Canopy Width Measurement

1. Select an average-size plant and draw a line to the canopy in the widest direction using the “Straight Line” tool.

2. Go to “Analyze > Measure” or Ctrl+M (Command+M on Mac) to perform the measurement. The data will be displayed as length.

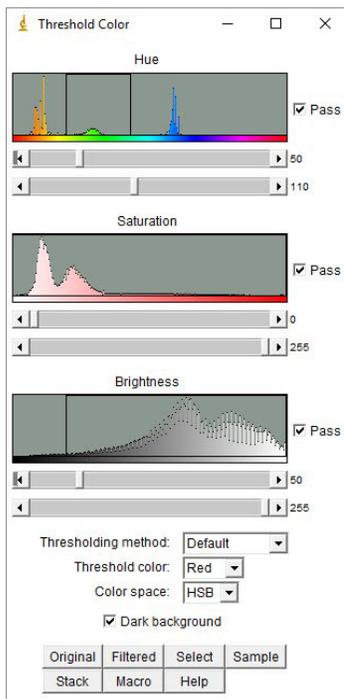
Canopy Cover Measurement

1. Go to “Image > Adjust > Color Threshold” to separate the canopy from the background.

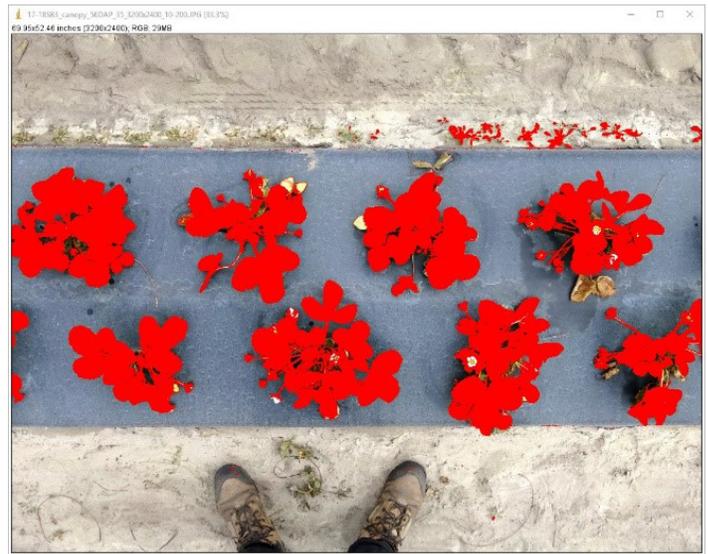
i. Adjust color threshold parameters (hue, saturation, and brightness) to select green color pixels.

Notes:

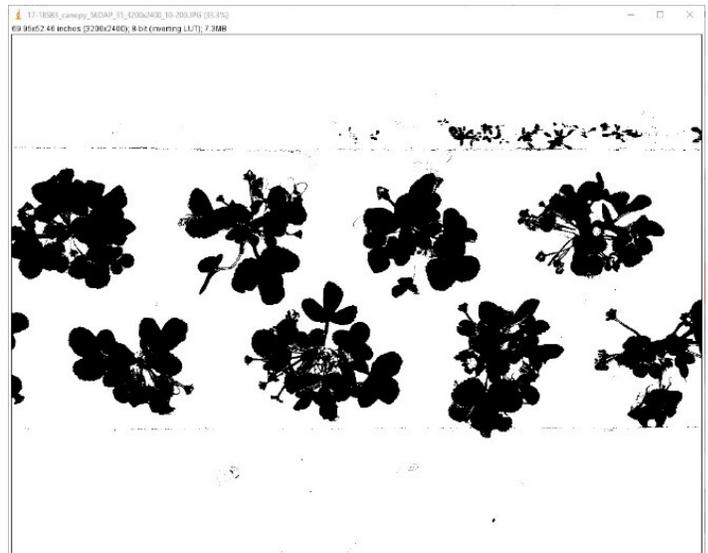
- Hue is one of color appearance parameters, and it describes pure spectrum colors. The hue range of 50 to 110 works well for most images.
- The optimum values can vary depending on the image quality, particularly for saturation and brightness.
- For best results, images should be collected under overcast weather.



ii. Click on “Select.”

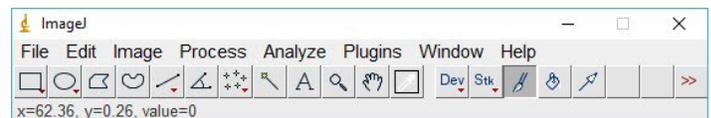


2. Go to “Process > Binary > Make Binary” to convert the color image to black and white.



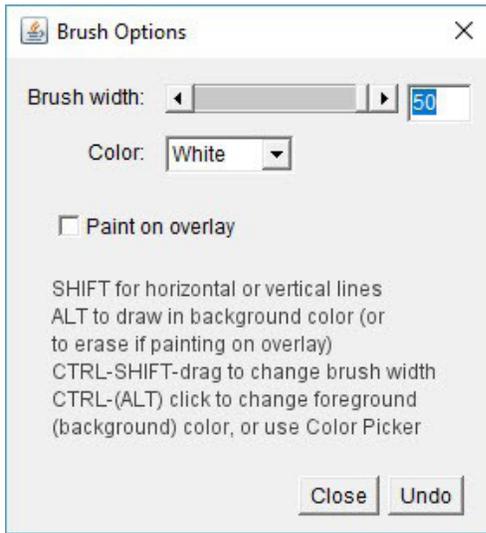
3. Use “Paintbrush Tool” to remove large clusters of pixels that do not belong to the object of interest.

i. Double click the “Paintbrush Tool” (highlighted below).

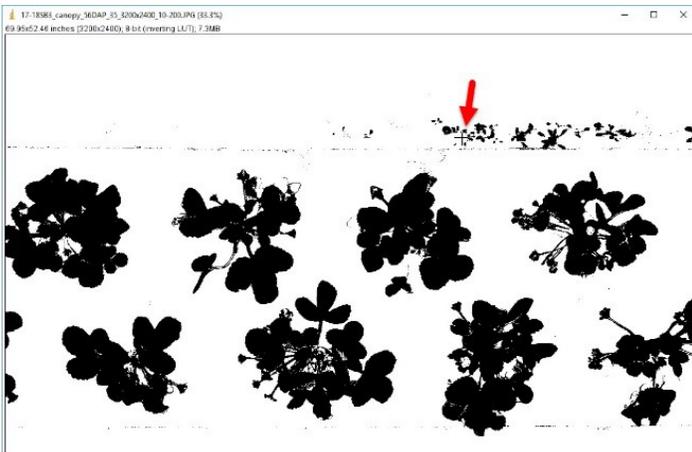


ii. Select the following parameters.

- Brush width: 50
- Color: White
- Paint on overlay: Unchecked



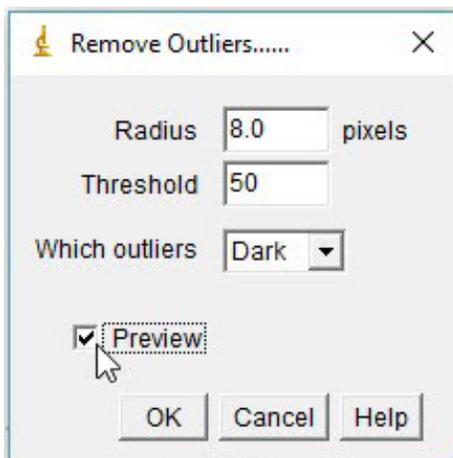
iii. Click on “Close” and erase the pixels that are not strawberry canopy (e.g., weeds).



4. Go to “Process > Noise > Remove Outliers” to remove small specks that do not belong to the object of interest.

i. Select the threshold values.

- Radius: 8
- Threshold: 50
- Which outliers: Dark



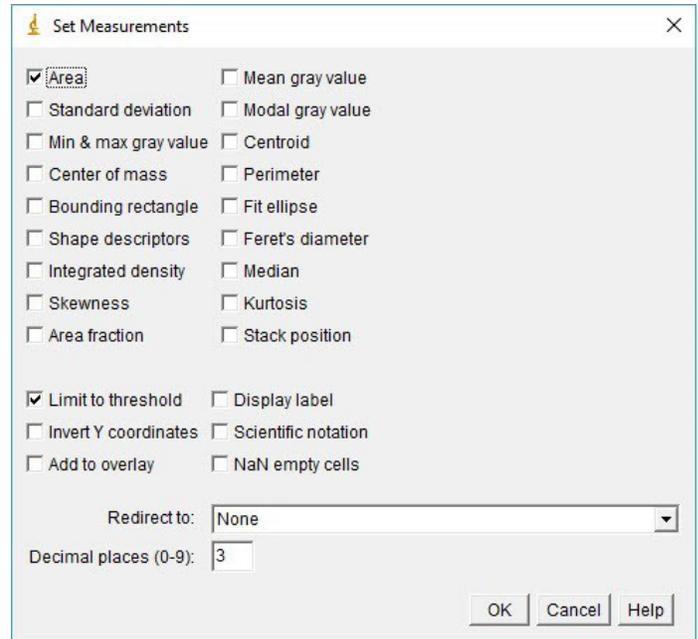
ii. Check on “Preview.” If everything looks good, click on “OK.”

Notes:

- Increase the radius if outliers are not removed.
- Select the radius that can remove outliers without deleting a significant portion of the canopy.

5. Select measurement variables and perform measurements.

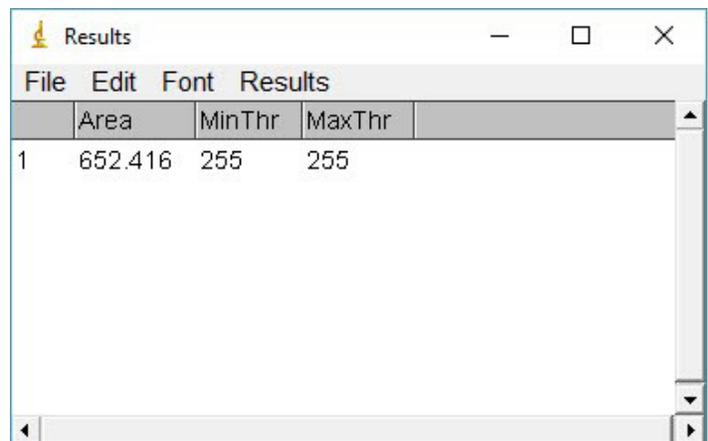
i. Go to “Analyze > Set Measurements” and select “Area” and “Limit to threshold.”



ii. Click on “OK.”

iii. Go to “Analyze > Measure” or Ctrl+M (Command+M on Mac) to perform the measurement.

iv. To determine the canopy cover per plant, divide the data by the number of plants in the image.



Literature Cited

Patrignani, A., and T. E. Ochsner. 2015. "Canopeo: A Powerful New Tool for Measuring Fractional Green Canopy Cover." *Agron. J.* 107: 2312–2320.

Sozzani, R., W. Busch, E. P. Spalding, and P. N. Benfey. 2014. "Advanced Imaging Techniques for the Study of Plant Growth and Development." *Trends in Plant Science* 19: 304–310.

Stewart, A. M., K. L. Edmisten, R. Wells, and G. D. Collins. 2007. "Measuring Canopy Coverage with Digital Imaging." *Commun. Soil Sci. Plant Anal.* 38: 895–902.