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A Simple, Inexpensive, and Portable Image-Based Technique for Nondestructive Leaf Area Measurements[1]

Shinsuke Agehara, Lillian Pride, Mariel Gallardo, and Jose Hernandez-Monterroza[2]

This article introduces a simple, inexpensive, and portable image-based technique for nondestructive leaf area measurements (Figure 1). It uses an imaging apparatus made with ordinary office supplies to obtain leaf images in greenhouse or field environments. Leaf images are then processed and analyzed to measure leaf area using ImageJ, an open-source image processing program. Because both image capture and analysis are performed nondestructively, leaf area can be measured on the same leaf repeatedly, enabling the monitoring of leaf growth over time. Leaf area data can also be used to assess leaf or whole-canopy physiology by converting the unit of other nondestructive measurements, such as photosynthesis and transpiration, from per-unit leaf area to per leaf. This technique is particularly useful to researchers and students studying leaf growth and physiology in greenhouse or field environments. This article is part of a series introducing various image-based measurements for horticultural research. The tutorial video for this article is available at the UF IFAS Horticultural Crop Physiology Lab YouTube channel (https://youtu.be/5JhH9-WosgA). Other ImageJ tutorial videos are also available at https://www.youtube.com/playlist?list=PL4qrjj3jZ6i568ToiUV-DvAsQ0Gyb30hK.

Figure 1. Capturing a pepper leaf image nondestructively in the field. The image apparatus in this photograph is described in Figure 3.

Introduction

Leaf area is an important horticultural trait that reflects plant health, growth, and environmental stress (Gonzalez et al. 2010). Leaf area data can also convert the unit of other

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2. Shinsuke Agehara, assistant professor, Horticultural Sciences Department; Lillian Pride, research technician; Mariel Gallardo, graduate research assistant; and Jose Hernandez-Monterroza, graduate research assistant; UF/IFAS Gulf Coast Research and Education Center, Balm, FL 33598.

measurements, such as photosynthesis and transpiration, from per-unit leaf area to per leaf, enabling the assessment of leaf or whole-canopy physiology.

Leaf area can be measured destructively or nondestructively. Traditional techniques such as grid count, paper weighing, and planimeter methods (Chaudhary et al. 2012; Montero et al. 2000) are rarely used in current horticultural research because of the time-consuming measurement processes. More modern techniques involve imaging leaves by scanners or cameras, followed by optical measurements or image analysis (Easlon and Bloom 2014). Various types of commercial leaf scanners are available. Stationary scanners with conveyor belts are used for destructive measurements, whereas portable palette- or wand-style scanners are used for nondestructive measurements. Their optical measurements are highly accurate. The major drawback of leaf scanners is their high instrument cost.

This article introduces a simple, inexpensive, and portable image-based technique for nondestructive leaf area measurements (Figure 1). The imaging apparatus is made with ordinary office supplies and designed to easily obtain leaf images in greenhouse or field environments. Leaf images are then processed and analyzed to measure leaf area using ImageJ, an open-source image processing program. The advantages and disadvantages of this technique are as follows:

Advantages

- The apparatus only requires inexpensive, ordinary office supplies. Also, ImageJ is a public domain program, and it is free to download.
- The image processing and analysis is quick—typically 1.5 to 2 minutes per image.
- The apparatus is portable and produces acceptable image quality for image analysis under many different environmental conditions (Figure 2).
- Measurements can be performed on the same leaf repeatedly, making it possible to monitor leaf growth over time.
- Images themselves are very good visual data.
- Photographs can be saved with metadata, such as geolocation, date, and time, automatically.

Disadvantages

- Leaf area measurements involve two separate steps: image acquisition and image analysis. Data cannot be obtained until images are analyzed.
- Using ImageJ for image processing and analysis requires some basic computer literacy and installation of a compatible version of Java software (https://www.java.com/ES/download/).

Figure 2. Hop leaf images successfully processed with ImageJ under three different weather and lighting conditions.

Taking Photographs

Imaging Apparatus

The imaging apparatus for capturing leaf images nondestructively is shown in Figure 3. It is assembled using the following items:

- Cellphone or digital camera × 1
- Red clipboard × 1
- Clear folder pocket × 1
- Magnets × 2
- Binder clips × 2
- Post-it (in this case 3 inches) × 1

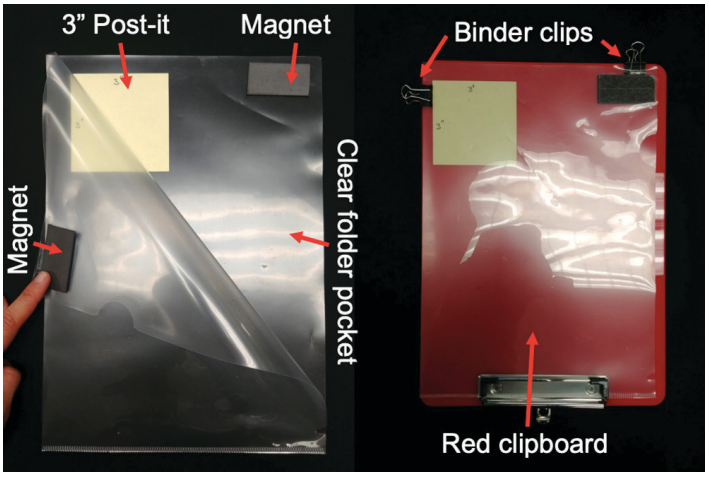


Figure 3. The imaging apparatus for capturing leaf images nondestructively. Left: A clear folder pocket with a magnet on each plastic sheet and a 3 inch Post-it inside. Right: A fully assembled apparatus. The upper-left binder clip should be clipped to the clipboard and the top sheet of the clear folder pocket. The upper-right binder clip should be clipped to the clipboard and the bottom sheet of the clear folder pocket.

Note:

- During the testing phase for this technique, different clipboard colors (e.g., white, red, black, brown) were used. We found that image processing was most successful when the red clipboard was used, probably because red is green's complementary color.

Image Capture Protocol

1. On the clear folder pocket, detach the magnet on the top sheet from the bottom sheet.

2. Place the leaf on the clipboard.

3. Reattach the magnets so the top sheet of the clear folder pocket is smooth and flattens the leaf.

Note:

- The leaf can slide out because it is held loosely by the imaging apparatus. Hold the apparatus still until image capture is complete.

4. Take a picture.

Note:

- Make sure the clipboard is perpendicular to the camera and all the corners are squared off. This ensures the scaled Post-it is the same focal distance from the camera as the leaf, and leaf area is not distorted by clipboard tilt.
- Choose an overcast day, or a camera angle that can minimize deep shadows in the image (e.g., a sunny day with no clouds at 12–2 p.m. for overhead plot photographs).
- Short of blinding glare, light reflection on the imaging apparatus will not affect image analysis.

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.

Note:

- Another download option is Fiji (https://imagej.net/Fiji/Downloads), a version of ImageJ bundled with many plugins.

Image Processing and Analysis

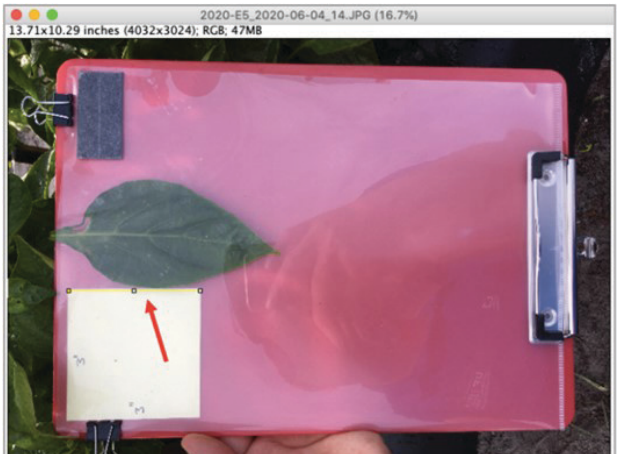
This example will use a photograph of a pepper leaf taken in the field. The photograph was taken using an iPhone 7 in the middle of a sunny day.

Step 1: Set Scale (Spatial Calibration)

1. Import the image: Go to "File > Open" and navigate to the image or drag the file onto the ImageJ toolbar.

2. Select the "Straight Line" tool (highlighted below).

3. Draw a line to an object of known size (e.g., Post-it).



Note:

- The image can also be scaled by the clipboard.

4. Go to "Analyze > Set Scale" to set the distance to the line.

   i. Enter the following parameters (e.g., Post-it size = 3 inches).

   - Known distance: 3
   - Unit of length: inch
   - Global: Unchecked

   ii. Click on "OK."

Note:

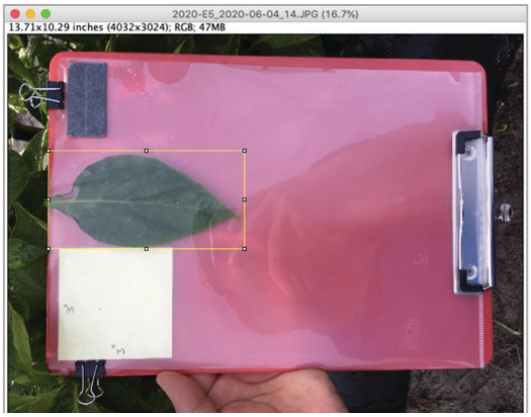
- When the "Global" option is checked, the same image calibration will be applied to images that will be imported subsequently. Use this option when the same image needs to be imported multiple times to perform different sets of image analysis or when all images in a set have the same scale. When working on an image with a different scale, "Set Scale" must be done again.

Step 2: Cropping the Image

1. Use the "Rectangle" tool to select the area of interest.

   i. Click the "Rectangle" tool (highlighted below).

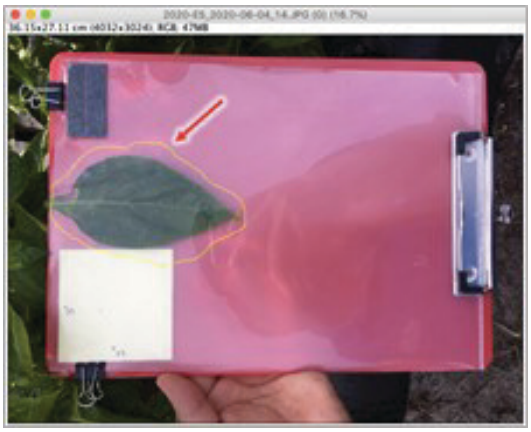
   ii. Select the smallest rectangle possible around the leaf.



2. Go to "Image > Crop" to crop the image.

Note:

- The magnet should be excluded from the cropped image because its hue and brightness are similar to those of the leaf. If it is impossible to exclude the magnet with the "Rectangle" tool, use the "Freehand selections" tool instead.



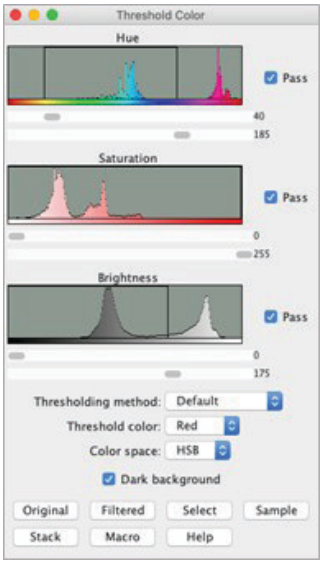
Step 3: Color Thresholding

1. Go to "Image > Adjust > Color Threshold" to separate the leaf from the background.

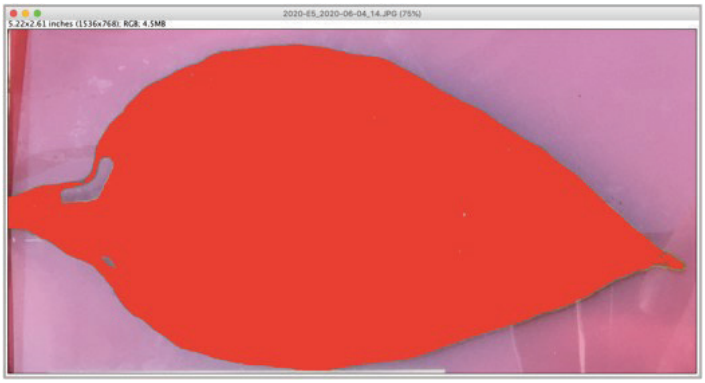
   i. Adjust color threshold parameters (hue, saturation, and brightness) to select the leaf.

Note:

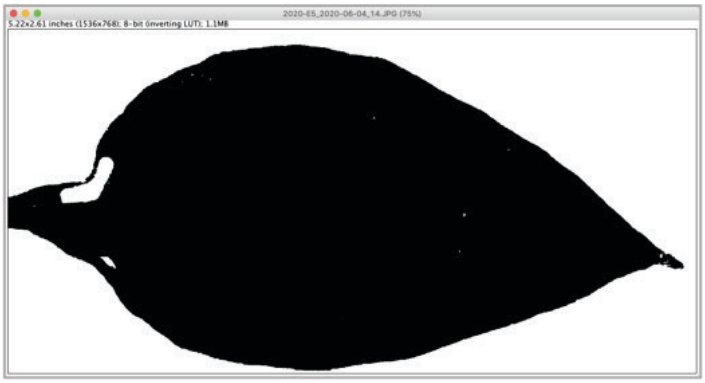
- Hue is one of the color appearance parameters, and it describes pure spectrum colors. Because red is green's complementary color, it is relatively easy to separate one from the other.
- In this case, hue range from 40 to 185, saturation from 0 to 255, and brightness from 0 to 175 works well.



   ii. Click on "Select."



2. Go to "Process > Binary > Make Binary" to convert the color image to a binary (black and white) image.

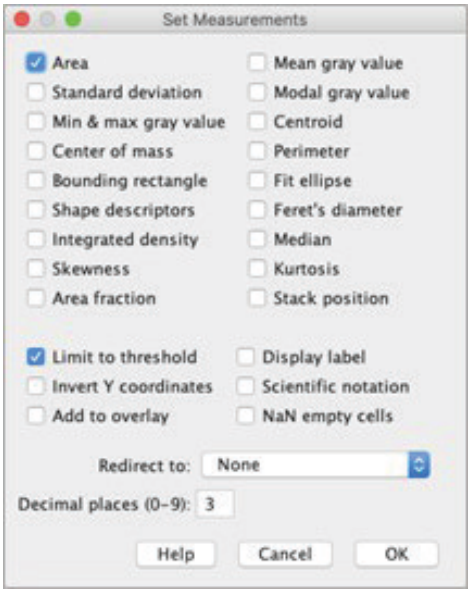


Step 4: Leaf Area Measurement

1. Go to "Analyze > Set Measurements" to select measurement variables.

   i. Select "Area" and "Limit to threshold."

   ii. Click on "OK."



2. Go to "Analyze > Measure" or Ctrl+M to measure the leaf area.

Leaf Area Estimation Accuracy

The accuracy of this technique was compared to the LI-3100C Area Meter (LI-COR, Lincoln, NE), which is one of the most commonly used instruments for destructive leaf area measurements. This instrument is equipped with two transparent conveyer belts that rotate to move leaves across a scanning bed, where light obstruction is measured to compute leaf area (Figure 4). The maximum resolution of leaf area measurements by this instrument is 0.1 mm^2.

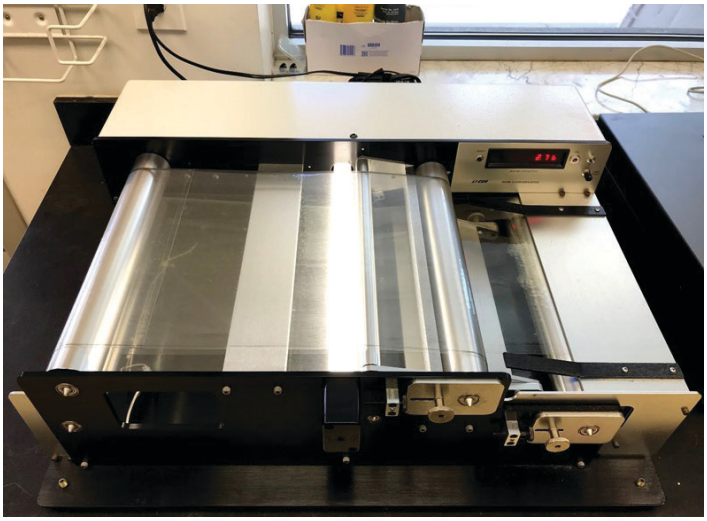


Figure 4. LI-3100C Area Meter (LI-COR, Lincoln, NE).

Leaf area was measured on 100 hop leaves and 100 pepper leaves by the LI-3100C Area Meter and the technique outlined above. The data were fit to a simple linear regression model: $y = ax + b$ (Figure 5). For both crops, the correlations were nearly 1:1 with extremely high r^2 values, demonstrating the high accuracy of the nondestructive leaf area measurement technique described in this article.

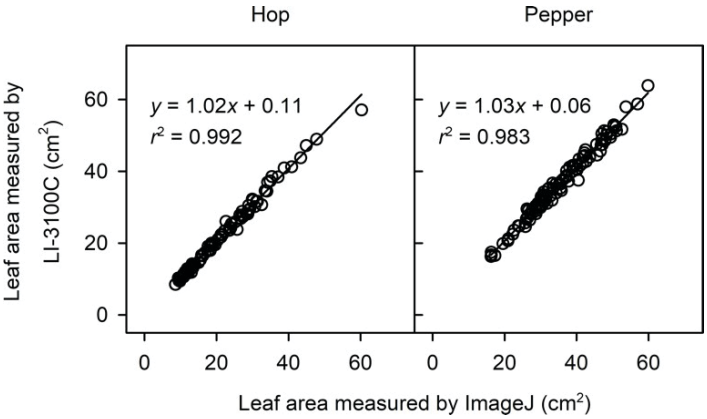


Figure 5. Linear regression analysis for 100 hop leaves (left) and 100 pepper leaves (right) between leaf area measured by the image analysis with ImageJ and by the LI-3100C leaf area scanner.

Other Image-Based Measurements

With the line tool in ImageJ, other leaf morphological traits, such as leaf length and width, can also be measured from the images collected by this method (Agehara 2020).

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