Development of an automated pigmentation phenotyping and low-cost multispectral imaging system

Changhyeon Kim[1,1], Kahlin Wacker[1,1], Benjamin Sidore[1,1], Tony Pham[1,1], Mark Haidekker[1,1], and Marc W Van Iersel[1,1]

[1]University of Georgia

November 30, 2022

Abstract

Canopy imaging is a good phenotyping approach to non-invasively quantify parameters such as canopy size, stress symptoms, and pigment concentrations. Unlike destructive measurements, canopy imaging is fast and easy. However, analysis of the images can be time consuming. To facilitate large-scale use of imaging, the cost of imaging systems needs to be reduced and the analysis needs to be automated. We developed low-cost imaging systems using a Raspberry Pi microcomputer, equipped with a monochrome camera and filter, at a total hardware cost of ˜$500. The latest version of our imaging system takes images under blue, green, red, and infra-red light, as well as images of chlorophyll fluorescence. Images taken under red, green, and blue light can be combined to generate color images. Other colors of light can be easily added, if desired. The imaging system is easily implemented in controlled environment agriculture and can be adapted for use in field settings. We will demonstrate examples of simple imaging techniques and automated image analysis using the Python programing language. The multi-spectral imaging system generates normalized difference vegetative index (NDVI) and anthocyanin content index (ACI) images and histograms, providing quantitative, spatially-resolved information.

Title of Paper: Development of an automated pigmentation phenotyping and low- cost multi-spectral imaging system

Reviewer Comments:

There are a couple of minor grammatical issues:

"... which utilizes seven colors of light emitting diodes (LED; 450, 521, 593, 625, 660, 730, and 870 nm) for acquiring multispectral images and a longpass filter (> 665 nm) to create mask image based on chlorophyll fluorescence."

... to create a mask image ...

"Differences chlorophyll levels between low and sufficient fertilizer treatments were evident from the average NDVI value of the plants and easily visualized in false color NDVI images (Figure 1)."

Differences in chlorophyll levels ...

In general, I think this short paper is easy to read and understand. The authors present a low-cost system for multispectral imaging using monochrome cameras and LEDs. They show significant correlations between their method and other approaches/measurements.

The methods seem to be appropriate for the objective and the imaging system is reasonably well documented.

There is no data or code availability statement, so I am unable to reproduce any results or examine code.

We are planning to write a manuscript with the data used in this proceeding. Therefore, we do not want to jeopardize our later manuscript by the proceeding.

I think that that introduction could be expanded. I was surprised that the abstract is nearly 100 words longer than the introduction. I think that the intro could benefit from more of a "...what do we know? What do we want to know? here we show..." format. I do not have a sense of the state-of-the-art in stationary multi-spectral phenotyping.

We edited the abstract and introduction to make clear what we want to present.

There is no description of the experiment design for the fertilizer experiment in the methods. In the results, there is a figure (Fig 1) but very little description of the finds of this experiment.

Again, we do not want to share the detail at this moment for later publication. On the later publication, we will share all the details including the codes

I might try to reframe the results and discussion as (1) the system, (2) the "calibration" experiments, (3) the fertilizer case study.

We changed structure of it as you suggested.

The conclusions did not leave me with a sense of the contribution to the state-of-the-art. It is well-known that images are sources of data and that R-Pi computers are useful tools. What does this study demonstrate and why should the readers be interested in this work?

We rewrote the conclusion as you suggested.

The proceeding is well written and presents a cost-effective imaging platform that will be of interest to the community. Given the focus of the proceeding it would be good to include links to the code that was used. The proceeding highlights cost-effective technologies that can be used to create accurate imaging and image analysis pipelines.

In general, we shorten some unnecessary sentences and rephrased points we would like to present, especially in introduction and conclusion.

Development of an automated pigmentation phenotyping and low-cost multispectral imaging system

Changhyeon Kim[a], Kahlin Wacker[a], Benjamin Sidore[a], Tony Pham[b], Mark Haidekker[b], and Marc W. van Iersel[a]
[a]Department of Horticulture, University of Georgia, Athens, GA, USA 30606; [b]College of Engineering, University of Georgia, Athens, GA, USA 30606

ABSTRACT

Canopy imaging is a non-invasive phenotyping approach to quantify parameters such as canopy size, stress symptoms, and pigment concentrations. Unlike destructive measurements, canopy imaging is fast and easy. However, analysis of the images can be time consuming. To facilitate large-scale use of imaging, the cost of imaging systems needs to be reduced and the analysis needs to be automated. We developed low-cost imaging systems using a Raspberry Pi microcomputer, equipped with a monochrome camera and filter, at a total hardware cost of ~$500. The latest version of our imaging system takes images under blue, green, red, and infra-red light, as well as images of chlorophyll fluorescence. The system uses a Python-based program to collect and analyze images automatically. The multi-spectral imaging system separates plant from background using the chlorophyll fluorescence image and generates normalized difference vegetation index (NDVI) and anthocyanin content index (ACI) images and histograms, providing quantitative, spatially-resolved information. We verified that these indexes correlate strongly with leaf chlorophyll and anthocyanin concentration. The low cost of the system can make this imaging technology widely available.

Keywords: Multi-spectral imaging, Phenotyping, Automated image analysis, NDVI, ACI, Chlorophyll, Anthocyanins

1. INTRODUCTION

Image-based phenotyping has experienced an increase in interest due to its simplicity and efficacy[1, 2]. Imaging can characterize traits in a non-destructive, rapid, and high-throughput manner[3]. In addition, imaging can eliminate sampling bias and allow a more representative measurement[4]. Many commercial systems exist for making these measurements, with varying degrees of adaptability and quality. However, the high cost of most imaging systems makes this technology inaccessible to many plant scientists. In addition, many plant scientists do not have the expertise to analyze images in a time efficient manner. Low-costs systems that automate image collection and analysis can facilitate widespread use of multi-spectral imaging. One of the challenges with image analysis is the separation of plant from background, because the algorithms to do so may depend on pigmentation and thus be cultivar- or species-specific. CFI provides a simple, but effective solution to this problem, since it captures fluorescence emitted by chlorophyll in the plant. CFI simply exposes the plant to blue light, while a longpass filter allows only the red/far-red chlorophyll fluorescence to reach the image sensor[5]. This approach is inexpensive and does not require complicated algorithms for background separation[6]. Here, we introduce our imaging analysis program and a low-cost phenotyping system that uses CFI and multispectral images using a Raspberry Pi for image collection and analysis. Our objectives were (1) to automate image analysis to separate plant from background and to quantify canopy pigmentation, (2) to validate results of analyses with independent pigment measurements, and (3) to develop a low-cost multi-spectral imaging system that can be customized for specific desired outputs, with flexible post-processing.

2. Materials and Methods

2.1 Automated image analysis. For automated multi-spectral image analysis, we wrote a program in Python (v. 3.8) using the OpenCV library (v. 4.5.4). In summary, (1) the program reads multispectral images in a folder, (2) makes a mask image based on the chlorophyll fluorescence image, (3) executes image math calculations using the pixel intensity of the spectral images to calculate index values for each pixel, (4) generates a foreground index image and histogram, (5) exports average, standard deviation, and canopy size to a csv file, and (6) moves on to next folder and repeats these steps. In the image math calculations, equations for normalized difference vegetation index [NDVI; $(R_{870} - R_{660}) / (R_{870} + R_{660})$][7] and anthocyanin content index [ACI; $(R_{660} - R_{521}) / (R_{660} + R_{521})$][7] were used, where R is intensity of the pixel, a measure of reflectance, and the subscript indicates the wavelength.

2.2 Pigmentation validation. To evaluate the ability of the system to quantify pigment concentrations, indices from imaging, readings from hand-held pigment content meters, and pigment extract were compared. For NDVI imaging, red lettuce (*Lactuca sativa*) 'Cherokee', green lettuce 'Little Gem', mizuna (*Brassica rapa* var. *japonica*), and spinach (*Spinacia oleracea*) 'Whale F1' were grown with different fertilizer levels, which induced a wide range of chlorophyll concentrations. For anthocyanins, red lettuce cultivars 'Rouxai', 'Rex', and 'Teodore' were used. Multispectral images of these leafy vegetables were taken periodically with the TopView system (ARIS, Eindhoven, Netherland), which utilizes seven colors of light emitting diodes (LED; 450, 521, 593, 625, 660, 730, and 870 nm) and a longpass filter (> 665 nm) to create a CFI-based mask image. Chlorophyll and anthocyanin content indices were measured using a portable content meter of anthocyanins and chlorophylls (ACM-200plus and CCM-200plus, OPTI-SCIENCES, Hudson, NH, USA). Concentrations of anthocyanins were measured by following a modified protocol of Lee, Durst and Wrolstad [8]. Regression analyses between indices from images, portable meters and pigment extractions were done using the R software.

2.3 Low-cost system. A low-cost imaging system prototype, using a Raspberry Pi (Raspberry Pi 4 model B, Raspberry Pi Foundation, Cambridge, UK), monochrome camera, blue, green, red, and infra-red LEDs, and a movable longpass filter was developed. The ability of this system to collect and analyze multi-spectral was evaluated.

3. Results and Discussion

3.1 Automated image analysis

Our program accurately separates plant from background, based on chlorophyll fluorescence images[9], creates false color NDVI and ACI images, summarizes the information in histograms and export summary data to a csv file (Figure 1, 2). NDVI and ACI information is based solely on canopy properties and ignores background. This differentiation between background and plant material strengthens the correlation between average NDVI values and CCI measurements, compared to NDVI measurements that combine canopy and background.

3.2 Pigmentation validation

Differences in chlorophyll levels between low and sufficient fertilizer treatments were evident from the average NDVI value of the plants and easily visualized in false color NDVI images (Figure 1). In the lowest fertilizer treatment, two peaks were observed due to senescence of an older leaf, which did not occur in plants grown with high fertilizer levels. The NDVI values derived from the image analysis showed an asymptotic relationship with chlorophyll content index (CCI) ($R^2 = 0.87$, $p < 0.0001$, n = 193).

Average values of ACI from the image analysis system and anthocyanin content index, measured using a portable meter, were positively correlated ($R^2 = 0.47$, $p < 0.0001$, n = 108). In a different study, the ACI values from image analysis were more strongly correlated with anthocyanin concentrations in leaf extracts ($R^2 = 0.83$, $p < 0.0001$, n = 50).

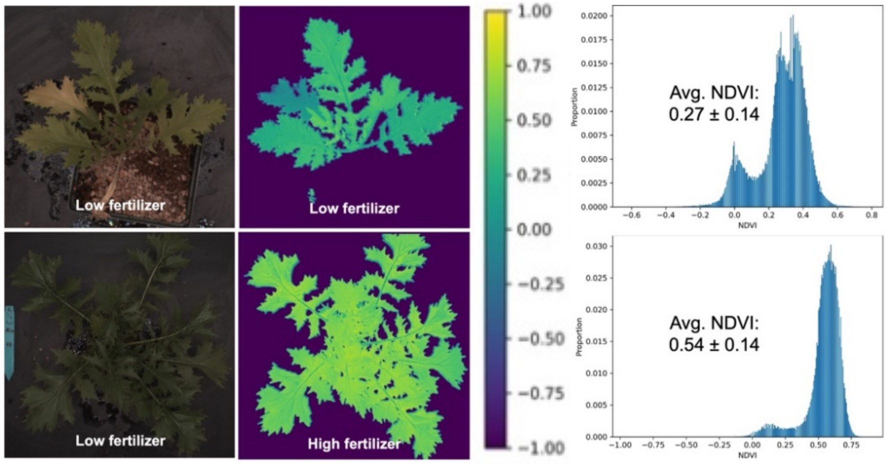

Figure 1. RGB (left) and Normalized difference vegetative index (NDVI) (middle) images of Mizuna under different fertilizer treatments and its corresponding histograms (right).

The greatest contrast in the optical properties of leaves due to the presence of anthocyanins is in the green part of the spectrum[10], but chlorophyll absorbs in the green part of the spectrum as well. Thus, the ACI also uses images taken under red light, where chlorophyll does and anthocyanins do not absorb, to normalize for the chlorophyll concentration. This approach is similar to NDVI imaging, where images taken under red light are indicative of chlorophyll levels, while infra-red images are used for normalization. ACI often is non-uniform throughout the canopy (Fig. 2), therefore, spot measurements of ACI may not represent the variability and distribution of anthocyanins throughout the canopy. However, the ACI imaging can visualize and quantify the heterogeneous anthocyanin distribution in the canopy.

Some limitations of this image-based phenotyping approach are associated with imaging from above. The images largely capture properties of cell layers near the top of the canopy. If anthocyanins are present on the abaxial side of leaves, the ACI imaging may not detect them. In addition, the camera can only see one layer of leaves and the system thus provides no information regarding lower leaves, if they are obscured by upper leaves.

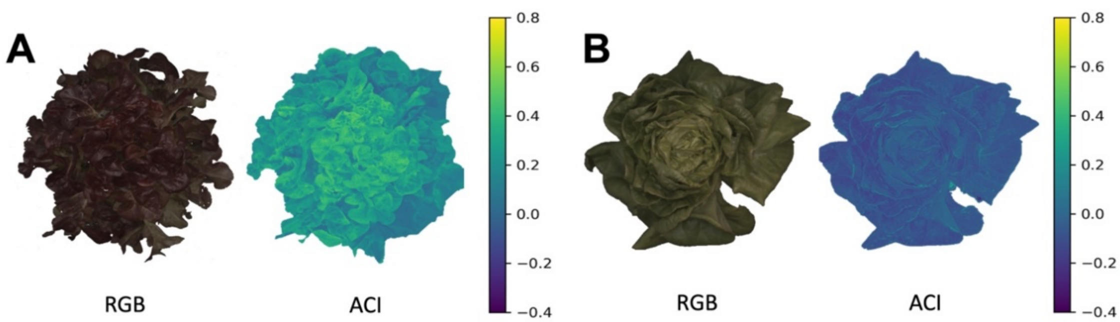

Figure 2. RGB and ACI images of ‘Rouxai’ (A) and ‘Rex’ lettuce (B). False color scale bar next to the ACI images corresponds to the ACI values of the canopy.

3.3 A low-cost imaging system using a Raspberry Pi

Commercially-available multispectral imaging systems are expensive and often do not have easily customizable post-processing capability. Given these factors, a low-cost multi-spectral imaging system was built using Raspberry Pi microcomputer (Raspberry Pi 4 model B) and utilizes the image analysis program we developed previously, which the Raspberry Pi can run natively in conjunction with the automated multi-spectral image capture (Figure 3 and 4).

The imaging station was installed in a light-proof grow tent, which was modified by adding a diffusing acrylic shelf near the top. LED strips (blue, red, green and near-infrared) were mounted on top of this shelf, pointed upward to create a diffuse light environment. A monochrome camera (UC-599 Rev.B, ArduCam, China) (Figure 3 D) was added, with the lens going through the acrylic diffuser. The camera module was enclosed in a 3D printed case, which includes a movable long-pass filter (> 665 nm) that can be placed in front of the lens. Exposing the plant to blue light induces chlorophyll fluorescence, which passes through the filter (Figure 3 C). This is a simple method to generate a mask image to separate plant from background. To collect this mask image, the program triggers a servo motor to place the filter in front of the camera. The filter is removed to take the other images. The Raspberry Pi uses relays to trigger the different colors of LED strips with the collection of monochrome images under each color of LEDs. The image collection and analysis can be scheduled using a native bash script and the results can be uploaded to the cloud.
The cost of the imaging system is ~ $500, including $60 for the Raspberry Pi, $50 for the camera, $50 for the LED strips, $240 of miscellaneous items (longpass filter, relays, monitor, keyboard, power supply, cases, wires, SD card, etc.), and a $100 growth tent. The software will be freely available. The strength of this system is that it can be customized to user needs. For example, facilities in controlled environment agriculture can set up this imaging system without the grow tent, in a pre-existing growth chamber.

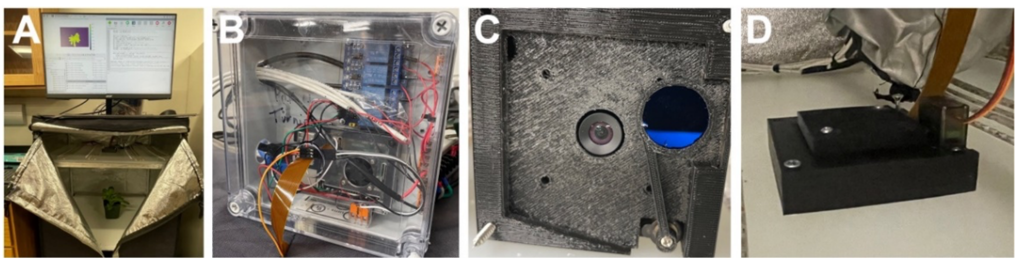

Figure 3. Overview of low-cost multi-spectral imaging system based on Raspberry Pi (A), wiring and relay array (B), detailed view of chlorophyll fluorescence filter system and bottom view of the camera module (C), and diffusion plate with LED strips and the camera module (D)

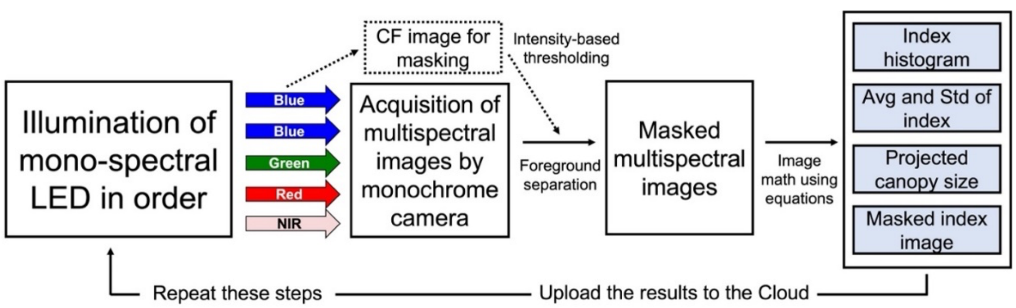

Figure 4. Schematic of the programing logic in the low-cost multispectral imaging system.

4. Conclusions

The usability of imaging systems can be improved by automated, customized post-processing. Multispectral canopy imaging can extract quantitative information related to anthocyanins and chlorophylls. Chlorophyll fluorescence imaging and its integration with a Raspberry Pi allowed for easy separation of plant from background, allowing further analysis of pixel that represent plant tissue only. The simplicity, low cost, and automated processing can make multi-spectral imaging available to a wide range of researchers and growers, who need such technology but cannot afford expensive systems.

ACKNOWLEDGMENTS

This project was funded by USDA-NIFA-SCRI Award Number #: 2018-51181-28365, project Lighting Approaches to Maximize Profits.

REFERENCES

[1] S. Das Choudhury, A. Samal, and T. Awada, "Leveraging Image Analysis for High-Throughput Plant Phenotyping," Frontiers in Plant Science, 10(508), (2019).

[2] W. H. Maes and K. Steppe, "Perspectives for Remote Sensing with Unmanned Aerial Vehicles in Precision Agriculture," Trends in Plant Science, 24(2), 152-164 (2019).

[3] C. Costa, U. Schurr, F. Loreto, P. Menesatti, and S. Carpentier, "Plant Phenotyping Research Trends, a Science Mapping Approach," (in English), Frontiers in Plant Science, Systematic Review 9(2019-January-07 (2019).

[4] J. C. Del Valle, A. Gallardo-Lopez, M. L. Buide, J. B. Whittall, and E. Narbona, "Digital photography provides a fast, reliable, and noninvasive method to estimate anthocyanin pigment concentration in reproductive and vegetative plant tissues," Ecol Evol, 8(6), 3064-3076 (2018).

[5] A. Mustafic, E. E. Roberts, M. D. Toews, and M. A. Haidekker, "LED-Induced fluorescence and image analysis to detect stink bug damage in cotton bolls," Journal of Biological Engineering, 7(1), 5 (2013).

[6] C. Kim and M. W. Van Iersel, "Morphological and Physiological Screening to Predict Lettuce Biomass Production in Controlled Environment Agriculture," Remote Sensing, 14(2), 316 (2022).

[7] S. Jacquemoud and S. Ustin, [Leaf optical properties], Cambridge, UK: Cambridge University Press, (2019).

[8] J. Lee, R. W. Durst, and R. E. Wrolstad, "Determination of Total Monomeric Anthocyanin Pigment Content of Fruit Juices, Beverages, Natural Colorants, and Wines by the pH Differential Method: Collaborative Study," Journal of AOAC INTERNATIONAL, 88(5), 1269-1278 (2005).

[9] S. Toda, K. Takayam, T. Kanoh, N. Fujiuchi, N. Takahashi, and H. Nishina, "Measurement of daily stem elongtation with chlorophyll fluorescence imaging robot," Eco-Engineering, 32(2), 15-21 (2020).

[10] A. A. Gitelson, M. N. Merzlyak, and O. B. Chivkunova, "Optical Properties and Nondestructive Estimation of Anthocyanin Content in Plant Leaves," Photochemistry and Photobiology, 74(1), (2001).