New Features and Many Improvements to Analyze Morphology and Color of Digitalized Plant Organs Are Available in Tomato Analyzer 3.0

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Abstract

Tomato Analyzer measures morphological and color attributes via image analysis in an objective, high-throughput, and semiautomatic manner. This software allows for reproducible quantification of phenotypic data that previously were done by hand or visual analysis. The new version has improved the accuracy of all measurements and reduced the need to make time-consuming manual adjustments. In this paper new morphological and color attributes available in Tomato Analyzer 3.0 as well as how the color test module was made more user-friendly are described.

Introduction

The species in the plant kingdom are characterized by a great diversity in color, shapes and size displayed in organs such as leaves, flowers, and fruits. Even within a particular species the individuals also can be distinguished by the morphology and color displayed in those organs. Biologists trying to understand the genetic and molecular basis for this variation need to measure morphological and color attributes in an objective and reproducible way. Most of this type of phenotypic analysis consists of time-consuming manual measurements or subjective visual scoring of characteristics that reduce the success of identifying genomic regions or physiological causes underlying this variation.

Tomato Analyzer (TA) is a software program designed to collect objective data from digital images obtained from plant organs (Brewer et al, 2006). Many of these data are nearly impossible to quantify manually, such as angles at the distal and proximal ends of the organs or the variation for color in their surfaces. Briefly, the software recognizes the objects (fruits, leaves or seed) in digitalized images and from the detected boundaries in each object is able to obtain more than 35 morphological attributes. The pixels inside the boundaries recognized by the software are used to translate color data from the RGB system into the L*a*b* universal color space which is able to approximate human visual perception (Darrigues et al, 2008). Moreover, TA combines controlled vocabulary consistent with terms present in trait ontology databases and mathematical descriptors for each shape and color attribute. Even though the application was specifically developed to analyze tomato fruit, this software can be applied to analyze fruit of other species and other plant organs such as seeds, flowers, and leaves.

This paper describes how morphological and color analysis of plant organs can be precisely done using Tomato Analyzer. Lastly, some possible applications of Color Test in Tomato Analyzer 3.0 are discussed.

1. Morphological Analysis of Plant Organs Using Tomato Analyzer

Tomato Analyzer can do a high-throughput analysis of morphological traits in images obtained from plant organs. Moreover, the data obtained are unbiased compared to those manually measured by different researchers or using different instruments. This impartiality allows the reproducibility of the experiments as well as the compilation and analysis of data obtained from experiments conducted in several environments and years.

To date, most of the morphological classifications in plants were made based on eyeball observations. Instead, attributes of TA can be used to objectively classify plant organs into various morphological categories.

Tomato Analyzer 2.0 has been a valuable and effective tool to indentify and confirm genomic regions that control tomato fruit shape as well as performing in-depth analyses of the effect of key fruit shape genes on plant morphology.

It was possible due to color of tomato fruits are contrasting enough with a black background used in the scanned images. However, the software was unable to detect other darker fruits or leaves.

1.1 Workflow for Morphological Analysis

- Scan plant organs against a black background to eliminate shadows.
- Open the image in TA, select the attributes to measure, tell TA to analyze the image.
- TA separates objects from background. It does this by looking at a histogram of luminance for the image and finding the separation point (area of low histogram values) between the foreground (lighter colors) and the background (darker colors). It then finds contiguous areas of foreground pixels (the objects) and calculates the boundaries around them.
- Resulting data appears in a spreadsheet panel in TA (screenshot) and can be exported as .csv file.
- Some measurements can be manually adjusted.

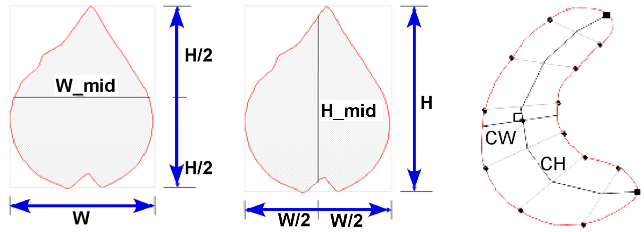
1.2 New Features and Attributes for Morphological Analysis in Tomato Analyzer 3.0

1.2.1 Reading TIFF images

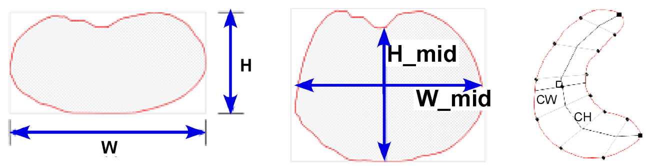
The previous version of Tomato Analyzer read only JPEG (.jpg) files but this new version can open both JPEG (.jpg) and TIFF (.tif) files. TIFF files are recommended because they preserve the image as it was originally scanned. JPEG images alter some of the colors in the image, reducing the accuracy of object boundary detection and color analysis. For example, this new feature has improved the boundary detection in dark green leaves and cucumber fruits. This improves the accuracy of all measurements and reduces the need to make time-consuming manual adjustments.

1.2.2 Length, width and fruit shape index attributes in curved fruits.

One of the most important features to analyze fruit shape in tomato is the fruit shape index (defined as the ratio between the width and the height of the fruits). When the tomato fruit or other type of fruits as cucumber is curved, the values for this index do not represent the actual value, they are underestimated. A new measurement, named curved height, was added in the new version of Tomato Analyzer (Figure 1). This attribute allows an accurate estimation of the length on curved fruits as well as is possible to be measured a new fruit shape index attribute.



- **Width Mid-height** (W_mid) – The width measured at ½ of the fruit's height.
- **Maximum Width** (W) – The maximum horizontal distance of the fruit.
- **Height Mid-width** (H_mid) – The height measured at ½ of the fruit's width.
- **Maximum Height** (H) – The maximum vertical distance of the fruit.
- **Curved Height** (CH) – The height measured along a curved line through the fruit (passing through the midpoints of opposing pairs of points on either side of the distal and proximal points).



- **Fruit Shape Index External I** (H / W) – The ratio of the Maximum Height to Maximum Width.
- **Fruit Shape Index External II** (H_mid / W_mid) – The ratio of Height Mid-width to Width Mid-height
- **Curved Fruit Shape Index** (CH / CW) – The ratio of Curved Height to the width of the fruit at mid-curved-height, as measured perpendicular to the curved height line.

Figure 1. Basic measurement attributes of Tomato Analyzer 3.0 and fruit shape index ratios based on this basic measurements.

1.2.3 Increased number of morphometric points

To measure shape without selecting individual attributes, TA offers a morphometric or geometric analysis of each object. This function finds points along the boundary of each tomato slice in the loaded image. Statistical tools such as Principal Component Analyses can be used to analyze the points in the exported data and it has been used to identifying tomato genome regions that control fruit morphology (Gonzalo et al, 2009). The distal and proximal ends are used as landmark points for every object (Figure 2). The number of points measured along the boundary is defined by the user and ranges from 4 to 200 in this new version of TA. The first morphometric point (1x, 1y) is always the proximal end point. The origin (0,0) of the coordinate system is located in the upper left corner of the rectangle defined by the Maximum Width and the Maximum Height.

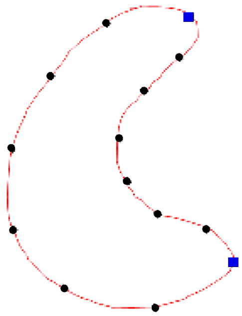


Figure 2. The morphometric points are a fixed number of points along the perimeter.

2. Color Analysis of Plant Organs Using Tomato Analyzer

Color is an important quality attribute in horticultural and floricultural crops defined by particular genes. However, color changes in plant organs also are indicators of biotic or abiotic stresses. The genetic bases for color attributes as well as factors affecting plant health would be better understood if computer-based analysis of digital images is applied instead of their subjective characterization. The Color Test module in Tomato Analyzer (TACT) is able of collecting and analyzing color parameters in an efficient, accurate and high-throughput manner from scanned images that contain plant organs. However, the scanner needs to be calibrated if the user intends to translate RGB values to L*, a* and b* parameters. This is because scanners may change in accuracy and how they capture the color scheme over time. Moreover, scanners differ in how well they capture color information.

2.1 Workflow for Color Analysis

- Scan a color checker. Open the image in TA and perform a color calibration.
- Scan objects and open the image in TA, as you would for morphological analysis.
- Select the color attributes to measure, and tell TA to perform color analysis.
- Resulting data appears in the spreadsheet panel, as in morphological analysis.

2.2 New Features and Attributes for Color Analysis in Tomato Analyzer 3.0

2.2.1 Color Attributes Visualized in real time

The most important improvement related to the color test module in this new version of TA is that the results are shown in the real time on the screen shot of the software (Figure 3). In the previous version, the results only could be visualized in .csv files after the analysis was done.

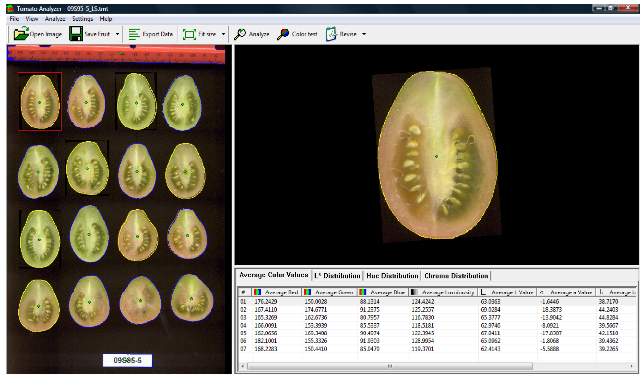


Figure 3. Screenshot of the Tomato Analyzer Test Color Module. This new version shows the color results in real time (right-down window).

2.2.2. A More User-friendly Test Color Module calibration

The user should chose a color checker with a black or very dark background based on the broad range of colors observed in the object of interest (Figure 4). Color checkers can be purchased custom made or standard.

	L*	a*	b*
1	37.986	13.555	14.059
2	65.711	18.13	17.81
3	49.927	-4.88	- 21.925
4	43.139	-13.095	21.905
5	55.112	8.844	-25.399
6	70.719	-33.397	-0.199
7	62.661	36.067	57.096
8	40.02	10.41	-45.964
9	51.124	48.239	16.248
10	30.325	22.976	-21.587
11	72.532	-23.709	57.255
12	71.941	19.363	67.857
13	28.778	14.179	-50.297
14	55.261	-38.342	31.37
15	42.101	53.378	28.19
16	81.733	4.039	79.819
17	51.935	49.986	-14.574
18	51.038	-28.631	-28.638
19	96.539	-0.425	1.186
20	81.257	-0.638	-0.335
21	66.766	-0.734	-0.504
22	50.867	-0.153	-0.27
23	35.656	-0.421	-1.231
24	20.461	-0.079	-0.973

Figure 4. Standard color checker from X-rite (Grand Rapids, MI) and actual L*, a*, b* values for each tile of the color checker are shown in the table.

After the color checker was scanned should be opened and analyzed in TA. The software recognizes each tile as an object. Then, the user needs to enter the actual L*,a*,b* values for each tile in the color checker, as provided by the manufacturer (Figure 4). TA uses the actual and observed L*, a*, and b* values to calculate the linear regression. After that, the color module is calibrated.

2.2.3 Three Methods to Analyze Color with Tomato Analyzer 3.0

- *Average color values.* The values displayed are: Average Red, Average Green, Average Blue, Average Luminosity, Average L* Value, Average a* Value, Average b* Value, Average Hue, Average Chroma. These average values are calculated taking account all pixel within the object.

- *L*, hue, chroma distributions.* These measurements provide histogram data for L*, hue, and chroma. The data appear in the tabs called L* Distributions, Hue Distributions, and Chroma Distributions, respectively. Each column shows the fraction of the object whose color falls within a certain range. For example, if the L[40..50) column in L* Distributions has the value 0.3, then 30% of the fruit has L* between 40 (inclusive) and 50 (exclusive).

- *Set custom color parameters.* Based on the L*, hue, chroma distributions, the user can define custom ranges of L*, hue, chroma, or a combination of the three. The User-Defined Color Ranges dialog appears as shown in Figure 5. The user can define up to 6 combinations of color ranges. In the example in Figure 5, Parameter 1 includes all colors where the hue is between 30 (inclusive) and 45 (exclusive); L* and chroma may be anything. Parameter 2 includes all colors where the L* is between 0 and 30 and the hue is between 0 and 90; the chroma may be anything. The data will appear in the data window tab called Custom Color Parameters.

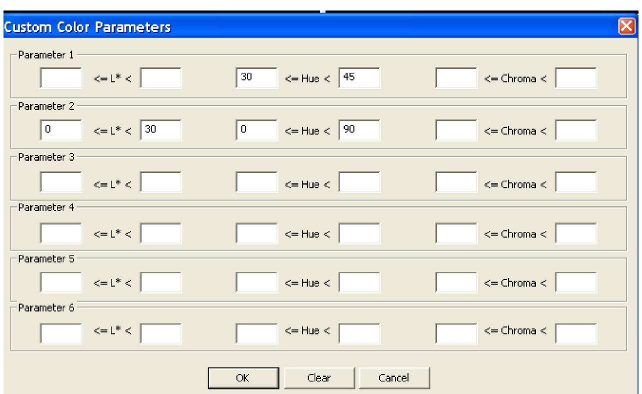


Figure 5. Set Custom Color parameters dialog box

2.3. Possible Applications Color Test Module of Tomato Analyzer 3.0

The Color Test module in Tomato Analyzer 3.0 is able to define the average for several color attributes inside the boundaries of a plant organ as well as the proportion of six different user-defined color parameters. This new feature can be useful to study pattern of color variation in some plant organ such as petals or leaves. In the plants, soil nutritional deficiencies, pesticides toxicities and even the severity of pathogen attacks affect the color pattern on some plant organ. For example, it has been demonstrated that the estimation of severity in a specific corn disease is highly affected by the rater experience when they directly estimate percentage of diseased leaf area and even more when they use a 0 to 9 ordinal rate scale (Poland and Nelson 2011). With TACT you can define a color range for the diseased portion and calculate the percentage of the leaf having that color. Thus, different researchers will get exactly the same results because they won't be interpreting the colors or the ratings differently. Therefore, Tomato Analyzer software would become in a powerful tool for this type of studies.

Acknowledgments

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