Tomato Analyzer User Manual Version 2.1.0.0

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Note: Tomato analyzer runs well on a Windows operating system.

Part 1: Basic Features

Image collection

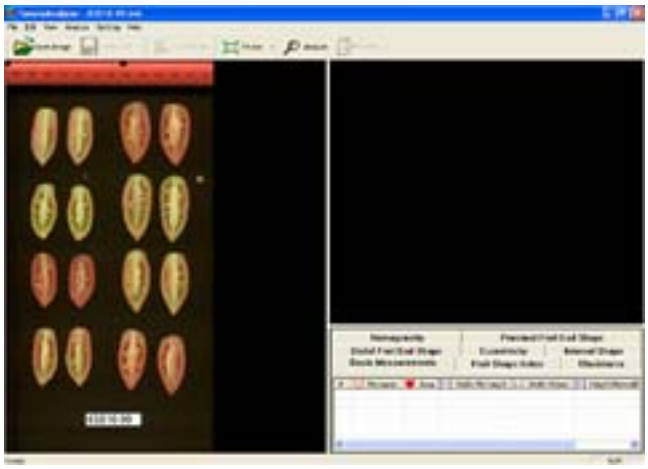
Objects should be scanned with a black background. This prevents shadows from interfering with the analysis. Dark objects, such as leaves, may need to be scanned with a white background for contrast, but shadows will need to be eliminated. The image also needs to be cropped during scanning or later with imaging software such as Photoshop. The Tomato Analyzer software will attempt to find objects throughout the image and if most of the image is empty (*i.e.* no objects), the software will not work properly.

The software is sensitive to image resolution and object size. Most fruits from 1 cm to 8 cm can be scanned and analyzed at 300 dpi (pixels/inch). If the fruits are large (> 8 cm), the resolution of the original image should be adjusted to approximately 100 dpi or smaller. The resolution of the original image needs to be set to approximately 750 dpi or larger in order to analyze very small objects, such as seeds. For accurate analysis, the dpi setting on Tomato Analyzer needs to be set to the same value as the images. See "Settings: scanner dpi" on page 10 of this manual.

Opening images

Start the Tomato Analyzer program and click on the "open image" 

button. Select and open the image file from the pop-up dialog box. The selected image will be displayed in the left window.



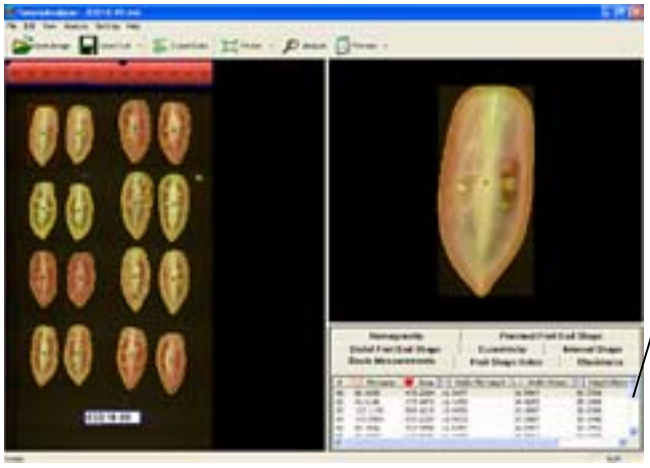
Automatic analysis

Set the dpi and measurement units to the appropriate settings by selecting "settings" and "scanner dpi" from the menu. Otherwise, the settings chosen by the previous user will remain in effect. The dpi setting needs to be the same as the image file so that the size measurements (height, width, perimeter, area) are accurate. The units measured setting determines the units (cm, mm, in, or pixel) for the data output. Prior to analysis, the user must select the attributes to be measured. See "Settings: Measurement

saved" on page 10 of this manual. Also, if blockiness, triangle, distal end angle are being measured, the user can choose settings for these attributes. See "Settings: Blockiness position and Macro/micro level" on pages 11-12 of this manual. The settings for blockiness, triangle, and distal end angle will also be kept at the settings chosen by the previous user.

To analyze the opened image, click the "Analyze" 

button. When finished, the perimeter of each fruit will be highlighted with a yellow line and the data will be displayed in the lower-right data window. The software will automatically deselect very large or small objects, such as a ruler or label. Additional fruits can be deselected by right clicking on them. Only items outlined in yellow will be displayed in the data window or exported. Items outlined in blue will not be included in any analyses. Individual fruits are displayed in the upper-right window by left-clicking on them with the mouse.



Save function

Click on the "Save Fruit" 

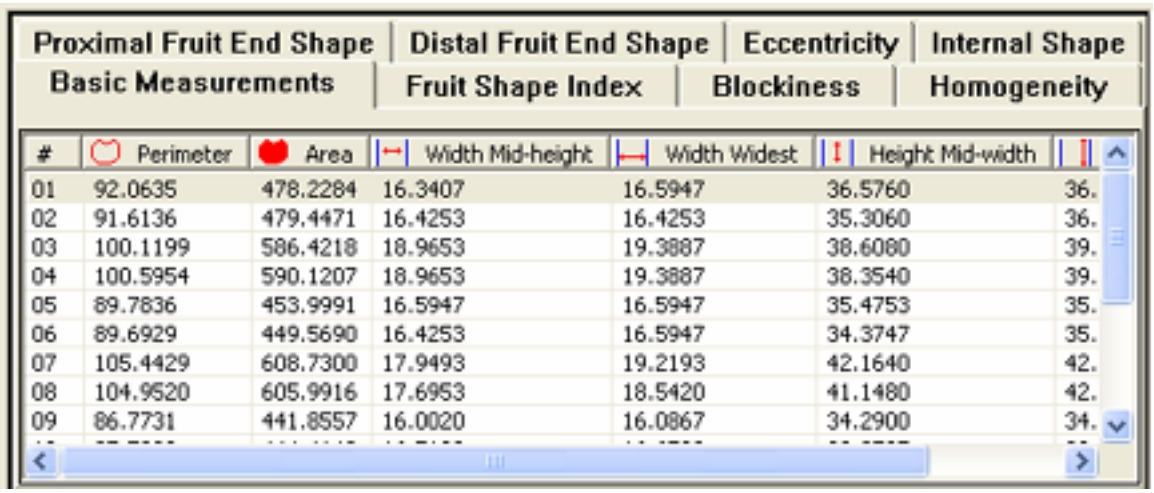
button. All current information, including manual adjustments and deselected objects, will be saved as a TMT file. The name of the file is the same as that of the original image except it has a different extension (tmt). Click the arrow on the right side of the "Save Fruit" button to select a file name different from that of the original image. Every time a file is selected, the saved tmt file with the same name will automatically be opened because it is linked to the original file. To revert to the original image file, without any of the adjustments, simply delete the associated TMT file. An alternative is to select "Analyze" to reanalyze the original image. However, beware that any tmt with the same name as the image will still be associated with the image the next time that the file is opened unless, of course, adjustments are saved over the tmt.

Results and data window

Shape attributes are divided into seven groups: Basic Measurements, Fruit Shape Index, Blockiness, Homogeneity, Distal Fruit End Shape, Proximal Fruit End Shape, and Eccentricity. By default, the "Basic Measurement" group is displayed. To view another group, click on the tab of the corresponding group. For detailed explanations of shape attributes, see the measurement reference document, "Tomato Shape Analysis".

Each row of data in the window corresponds to a particular fruit. The data is displayed in the same order as the objects in the image. The first row displays the values of the object in the upper left hand corner and the last data row displays the values of the object in the lower right corner of the image. Click on a row to display the corresponding fruit in the top window on the right. Alternatively, click on a fruit and the corresponding data row will be highlighted.

By clicking on the attribute tab in the lower-right window it is possible to view how that attribute is being measured for each particular fruit. For example, by clicking on distal end angle, the angle measured by the software for all of the fruits in the left window will be shown. This feature is very useful when identifying objects that require manual adjustments.



#	Perimeter	Area	Width Mid-height	Width Widest	Height Mid-width	
01	92.0635	478.2284	16.3407	16.5947	36.5760	36.
02	91.6136	479.4471	16.4253	16.4253	35.3060	36.
03	100.1199	586.4218	18.9653	19.3887	38.6080	39.
04	100.5954	590.1207	18.9653	19.3887	38.3540	39.
05	89.7836	453.9991	16.5947	16.5947	35.4753	35.
06	89.6929	449.5690	16.4253	16.5947	34.3747	35.
07	105.4429	608.7300	17.9493	19.2193	42.1640	42.
08	104.9520	605.9916	17.6953	18.5420	41.1480	42.
09	86.7731	441.8557	16.0020	16.0867	34.2900	34.

Export functions

There are two different export functions available with the Tomato Analyzer software. Both export the data from the analysis to an Excel file. The first method exports every value for every attribute of each object from an individual image. This method includes a calculation of the average and standard deviation. This method is useful for determining the variation within an individual plant. The second method is a batch analysis that averages the values of the fruits in a single image for each attribute. Several images can be selected simultaneously to be batch analyzed and all of the data will be exported to a single file. This method is useful for whole population studies, including QTL analysis.

1. **Individual images**

The "Export Data" function is used to export all of the data from one image. The values of each fruit, as well as the mean and standard deviation of each attribute for that image, are exported to an Excel file. Click on the "Export Data" 

button. All of the data displayed in the data window will be exported to an Excel file. The user specifies the location and name of the Excel file.

2. Batch analysis

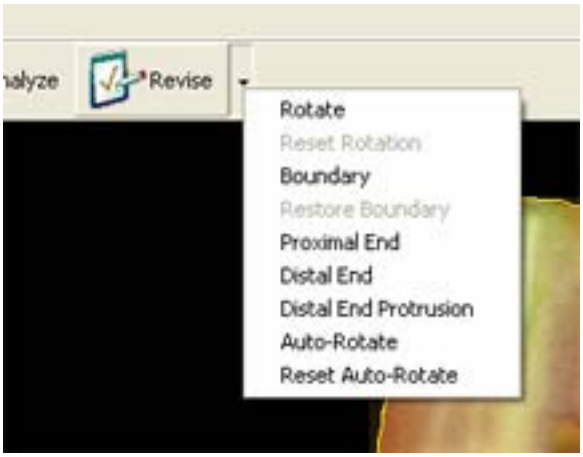
The "Batch Analysis" function is used to export the average values of each attribute for a user-selected amount of images. Click on the "Open" button. Select the files to be batch analyzed. Multiple files are selected using the "Shift" or "Ctrl" key while selecting additional files. After the files are selected, click on the "Open" key. Choose a name for the Excel file that will be created and click on the "Save" key. The software will automatically open the files and begin the batch analysis.

If the image files have been previously analyzed and saved by Tomato Analyzer, the saved files (tmt) will be opened for the batch analysis. If the files have not been previously analyzed, the software will perform the analysis with the original images. The software currently works best when analyzing up to 100 files in one batch. More can be analyzed depending on the number of objects in each image. However, be aware that the software may crash if too many images have been chosen for batch analysis. If this happens, select fewer files in subsequent analyses.

Part 2: Advanced Options

Manual adjustments

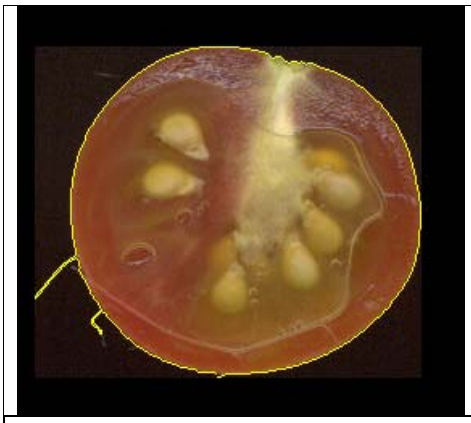
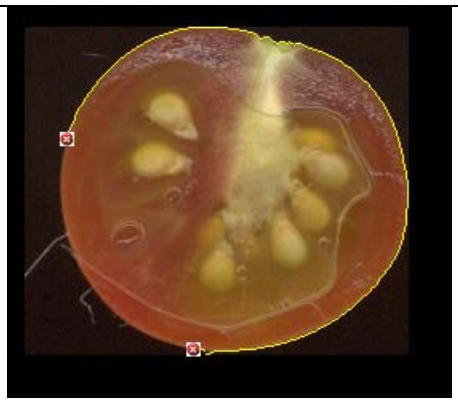
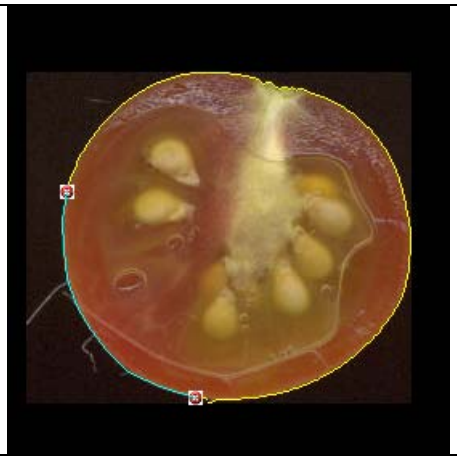
Manual adjustment allows for the correction of any errors in the automatic analysis. These adjustments are listed under the "Revise" tab. There are six types of manual adjustments: Rotate, Boundary, Proximal End, Distal End, and Distal End Protrusion and Autorotate. **Note: the software functions best if the boundary is adjusted prior to any other manual adjustments. The rotation should be adjusted, if necessary, prior to adjustment of the distal and proximal ends.**



1. **Boundary**

   This feature is useful when the fruit boundary contains errors. To adjust the boundary:

   a) Click on the fruit in the left window. It will appear in the upper right window.
   b) Click the arrow next to the "Revise" button and select "Boundary" from the drop down list
   c) In the upper-right window, select the boundary location that needs to be modified. This is accomplished by left clicking on the start point and end point of the incorrect boundary. As a result, the delimited boundary will be removed (see figure below).
   d) To add a new boundary, left click from the start point toward the end point. Continue clicking to follow the desired contour. Right clicking will undo a previous modification. Multiple modifications can be undone by consecutive right clicks (see figure below).
   e) To confirm new boundary, press the "Enter" key. Otherwise, press the "Esc" key to cancel this operation.
   f) Click on "Restore Boundary" to return to the original boundary setting.

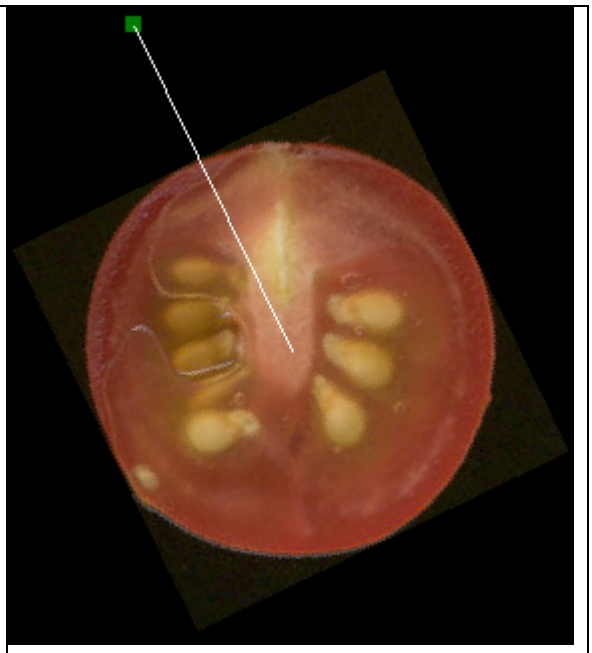
	
Original boundary	Select the segment of the boundary that needs to be modified.
	
Add new boundary	Adjusted boundary

2. **Rotate**

   This feature is used when a fruit image is not completely vertical.

   a) Click on the appropriate fruit in the left window. It will appear in the upper-right window
   b) Click the arrow next to the "Revise" button and select "Rotate" from the drop down list.
   c) An axis will be displayed in the upper-right window. Drag the green square at the end of the axis and the fruit will rotate accordingly.
   d) Double click inside of the window or press the "Enter" key to finish.
   e) Click on "Reset Rotation" to return to the original alignment.

Original image



After rotation

3. **Proximal end**

If there is an error with the position of proximal end, the proximal end measurements may be measured incorrectly. When the location of the proximal end is changed, the features at this end will be measured again automatically. To change the position of the proximal end:

a) Click on the desired fruit in the left window. It will appear in the upper right window.
b) Click the arrow next to the "Revise" button and select "Proximal End" from the drop down list.
c) In the upper-right window, click and drag the vertex indicator to the correct position.
d) Double click or press the "Enter" key to finish.

4. **Distal end**

The position of the distal end of the object can also be changed manually. To change the position of the distal end:

a) Click on the desired fruit in the left window. It will appear in the upper-right corner.
b) Click the arrow next to the "Revise" button and select "Distal End" from the drop down list.
c) In the upper-right window, click and drag the vertex indicator to the correct position.

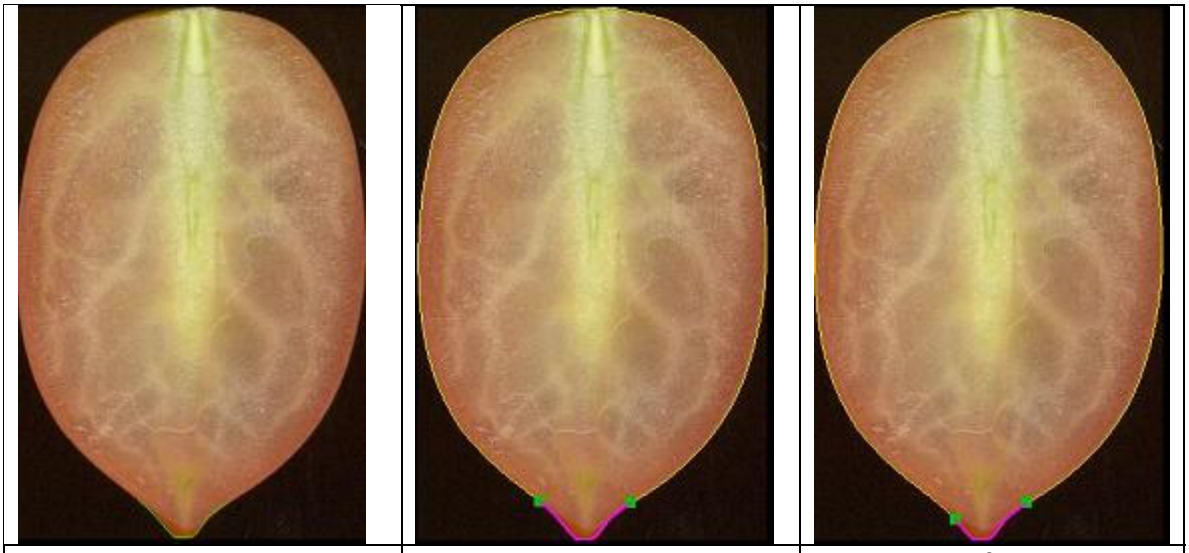
d) Double click or press the “Enter” key to finish.

5. **Distal End Protrusion**

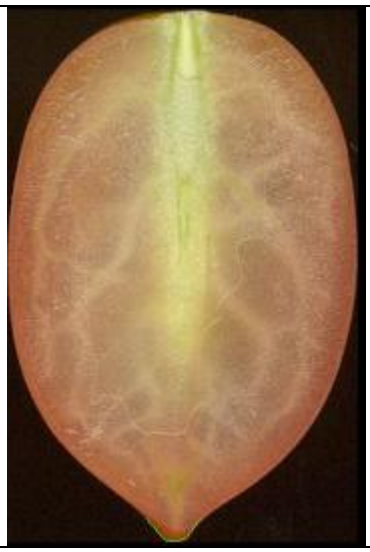
This feature is under construction.

At this time, this function is not working properly on all images. The position of the distal end protrusion can be adjusted by changing the left and right endpoints. To change the endpoints:

a) Click on the desired fruit in the left window. It will appear in the upper-right window.
b) Click the arrow next to the “Revise” button and select “Distal End Protrusion” from the drop down list.
c) Click and drag the left or right endpoint to the correct position.
d) Press the “Enter” key to finish.



Original image	Click “Revise” –“Tip area”	Adjust left endpoint

	
Adjust right endpoint	Press the “Enter” key to finish

6. **Autorotate**

This function is specifically developed for tomato seed such that they can be aligned with the pointed end facing the same direction automatically. At this time, autorotate function does not work on other objects such as fruit.

Settings

Settings can be changed by selecting “Settings” from the menu.

Scanner DPI:

The dpi value of the scanned image and the unit of the measurement can be adjusted by entering a new dpi value or selecting a new unit. Units include pixels, centimeters, millimeters, and inches and refer to output data values that are size measurements, such as area, perimeter, length and width. The default settings are 300 dpi and pixels. The brightness can be adjusted if the objects of the image are too similar in color to the background. This will make it easier for the software to accurately determine the boundary.

Measurement saved:

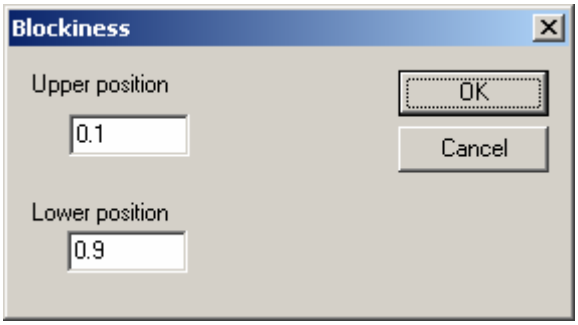
The traits that are measured and displayed in the data window, saved in the data export file (csv), or saved in the batch analysis can be selected. Individual attributes or an entire measurement group can be selected or deselected by clicking on the group or attribute. All attributes of a measurement group can be shown by clicking on the "+".

User-defined Settings

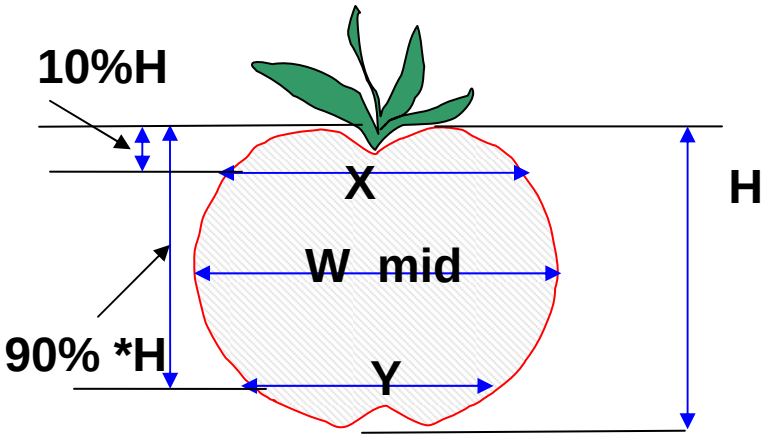
User-defined settings can be changed by selecting "Settings" from the menu.

Blockiness position:

The upper and lower blockiness positions can be changed by entering a new value. The default settings are 0.1 and 0.9 for the upper and lower positions, respectively.



The upper position is used for calculating proximal end blockiness and triangle. The setting for "Upper position" determines where X (see figure below) is measured. The lower position is used to calculate distal end blockiness and triangle. The setting for "Lower position" determines where Y (see figure below) is measured. In the figure below, upper positon = 10% and lower position = 90%. The values for these positions equal the percentage of the height from the top of the fruit.



Macro/micro level:

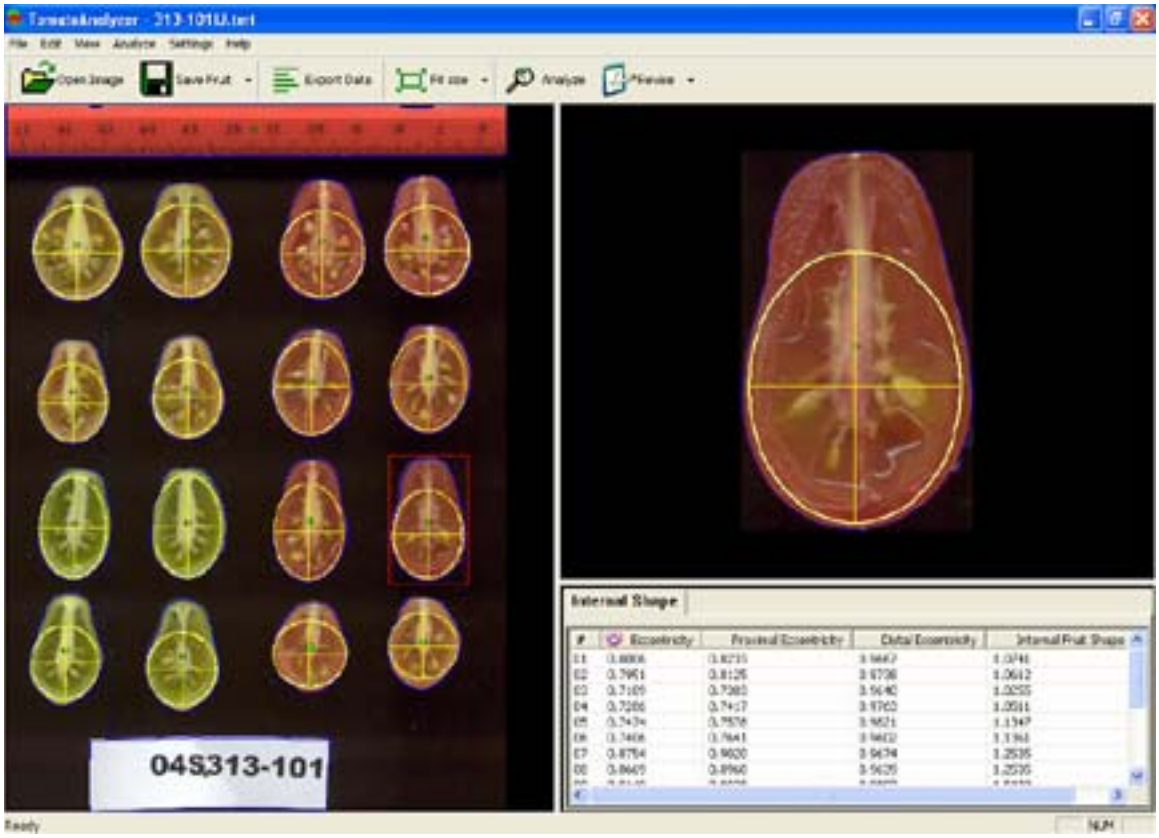
These settings are used for calculating angles at the distal end of the fruit. The setting for macro level determines the percentage of the perimeter from the bottom where the angle will be measured, typically ranging from 5-40 %. The software will determine the slope using +/- 5% of the selected value. The micro level setting determines where the proximal angle is measured, typically 2-10% from the tip of the fruit. The software will determine the slope using +/- 1% of the selected value.

Additional manipulations

These features are under construction.

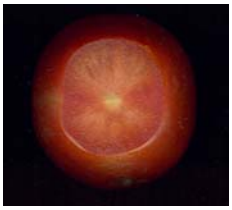
Internal Shape:

Subtle shapes are difficult to capture with a mathematical formula. The software contains a function that measures a user-implemented superimposed feature. This feature would be a line, square, or ellipse that delineates an internal structure. Measurements are made based on the user-implemented feature. These measurements are under the Internal Shape tab. Scanned images can be opened first in NIH-image software (http://rsb.info.nih.gov/nih-image/Default.html) to draw the internal feature. The internal feature must be drawn within the outer boundary of the fruit. We chose to superimpose an ellipse on fruit in our studies that required analysis of the internal seed cavity shape to determine obovate shape. The ellipse is drawn at 2 pt thickness and colored in white. The images are saved as JPEG, which can be opened in Tomato Analyzer for further analysis.



Color analysis

This feature was developed to assess fruit color and color uniformity of cut tomato. To analyze color uniformity, the percentage of red and white area can be calculated using the color test feature in the "settings" tab. Tomato Analyzer will check each pixel of the tomato slice to see if it is "red" or "white". The color of the pixels is measured using both the RGB and HSL color scales. In order for a pixel to be red, the pixel should have a low hue value. The user needs to define this range, which is [0, hue], where *hue* is the highest hue value that is considered to be red. Likewise, the user needs define what makes a pixel white by finding its luminosity value. A pixel that is "white" has a high luminosity value and the user needs to provide the range [lum, 240], where *lum* is the lowest luminosity value that is still "white". The border or skin of the fruit (see image below) should not be included in the calculation of red-white percentages. Therefore, the minimum blue value needs to be set so that only the cut surface will be analyzed for the red-white percentages. By default, minimum blue value is 0.



Color analysis output produces a CSV file with the average RGB, Hue and Luminosity values of each tomato slice, along with the percentages of the "white" and "red" area. Batch analysis can be used for color analysis also. If "batch" is checked in the "Settings, color test" box, Tomato Analyzer will prompt the user for selecting images for batch analysis. If batch is not checked, TA will analyze the current image and save it in a CSV file.