RootScan Users Manual






September 2012 Edition

About RootScan

RootScan is a semiautomated root cross-section image analysis program that allows users to detect and select different anatomical regions for future computational statistical operations. In addition to data collection, there are numerous options pertaining to image data and display that can be changed based on user preferences. The information contained in this manual should be used as a guide before, during and after any root cross-section analysis. New users should read this manual before they begin analyzing their root cross-section images.

For additional information about RootScan refer to the following paper:

Burton, AL, M Williams, JP Lynch, and KM Brown. 2012 RootScan: Software for high-throughput analysis of root anatomical traits. Plant and Soil 357:189-203, DOI: 10.1007/s11104-012-1138-2.

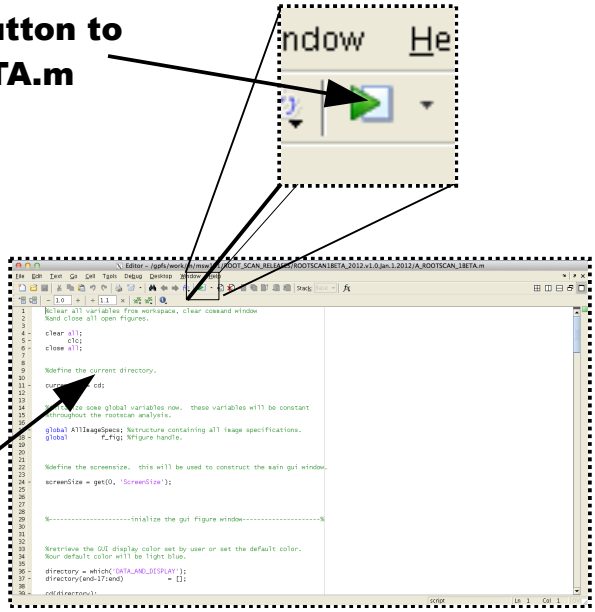
Disclaimer: Image quality is the major factor in determining overall data quality. With that said, the authors are not responsible for any data results.

Content

Getting Started with Rootscan

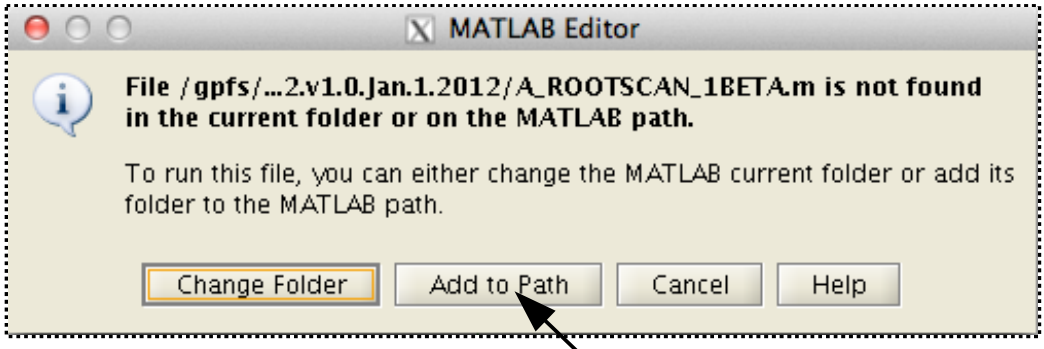
Rootscan was developed in the programming language “Matlab”. To get started, run the Matlab © application installed on your computer's applications folder or remotely using secure shell commands in the terminal. Once Matlab is initialized, **open** the main Rootscan program file 'A_ROOTSCAN_1BETA.m' and **run** the file.

Press the green play button to run A_ROOTSCAN_2BETA.m



A_ROOTSCAN_1BETA.m open in Matlab Editor window

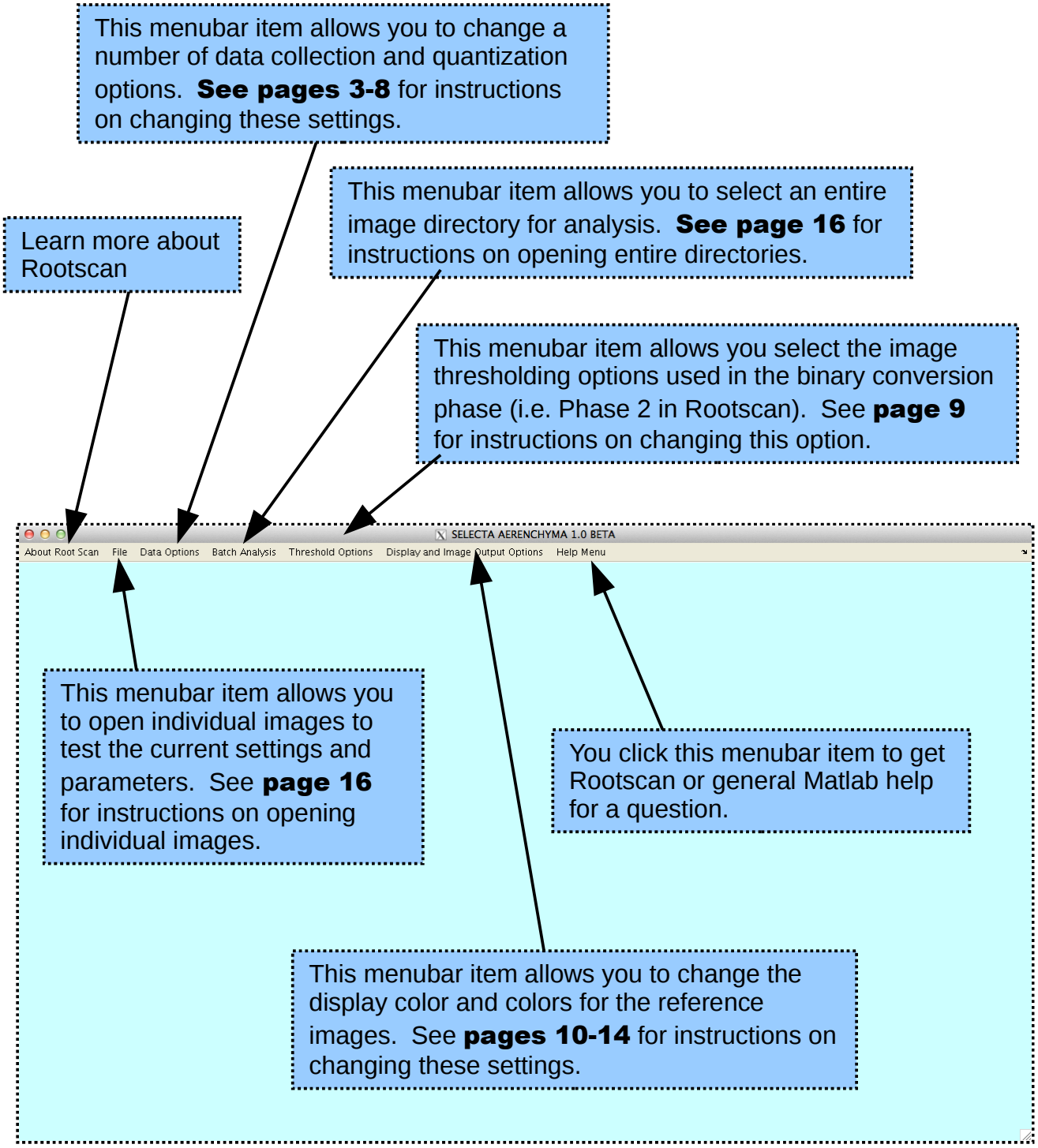
At this time you should see a dialog box titled: MATLAB Editor. This dialog box (seen below) informs you that your main Rootscan program file is not located in the current folder or on the MATLAB path. At this time, you can either change the MATLAB current folder or add your program files to the MATLAB path. **You should add your Rootscan folder to the MATLAB path at this time.** If you have MATLAB on your computer you can manually place the Rootscan programs folder in the MATLAB path. This action will negate the appearance of the MATLAB editor dialog box shown below. Once you selected the 'Add to Path' option the main Rootscan window will appear on your screen (see Page 2).



Click the 'Add to Path' option in the MATLAB Editor dialog box to run Rootscan during your MATLAB session.

The Main RootScan Window and MenuBar

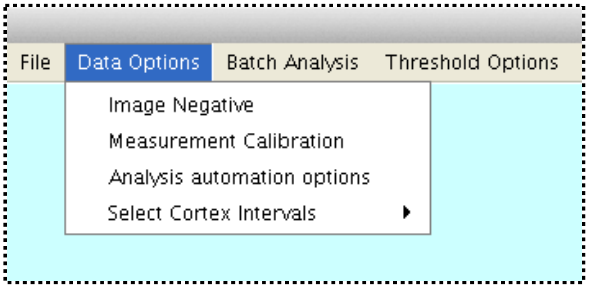
The main Rootscan window contains options determining what parameters and settings you want activated for image analysis and options for individual image and batch processing.



Changing Settings and Parameters

Setting the Data Precision and Pixel Calibration

The calibration and precision settings graphical user interface (GUI) is accessed directly from the main RootScan GUI window menubar under 'Data Options:Measurement Calibration' options. When this option is selected a separate window appears in the foreground of the main RootScan GUI. This window allows users to interactively change the pixel calibration and precision settings.



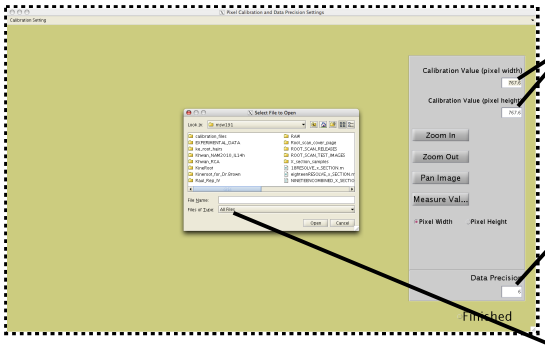
Setting the Data Precision Value

The data precision value is only set by direct entry. Users can do this by entering/reseting the current value in the data precision text box field. The bottom left image in the section: Setting the Calibration Value, shows the data precision text box field as it appears in the GUI.

Setting the Calibration Values

The pixel calibration value is essentially the value needed to convert from the pixel space to the metric space. You need the pixel calibration value, the pixels per linear millimeter, to perform this conversion. **NOTE: users need to measure two calibration values one for each dimension.**

The calibration value can be set using different methods. In the first method, the user has prior knowledge of the correct value and can enter the value in the text box under the calibration value header in the right hand side of the GUI window. If the value is unknown, users can measure the value directly by opening up their calibration file using the menu bar options in Calibration and Precision Settings GUI window.

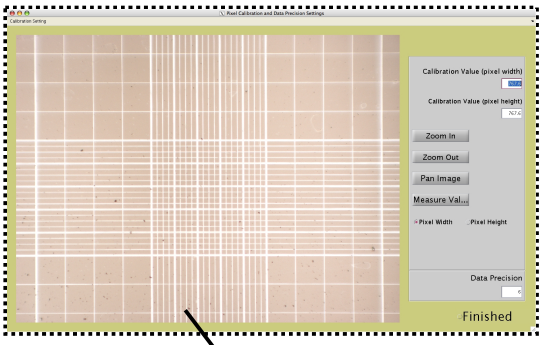


Calibration text boxes

Data precision text box

Selecting Calibration file under the calibration and precision settings GUI main menu bar

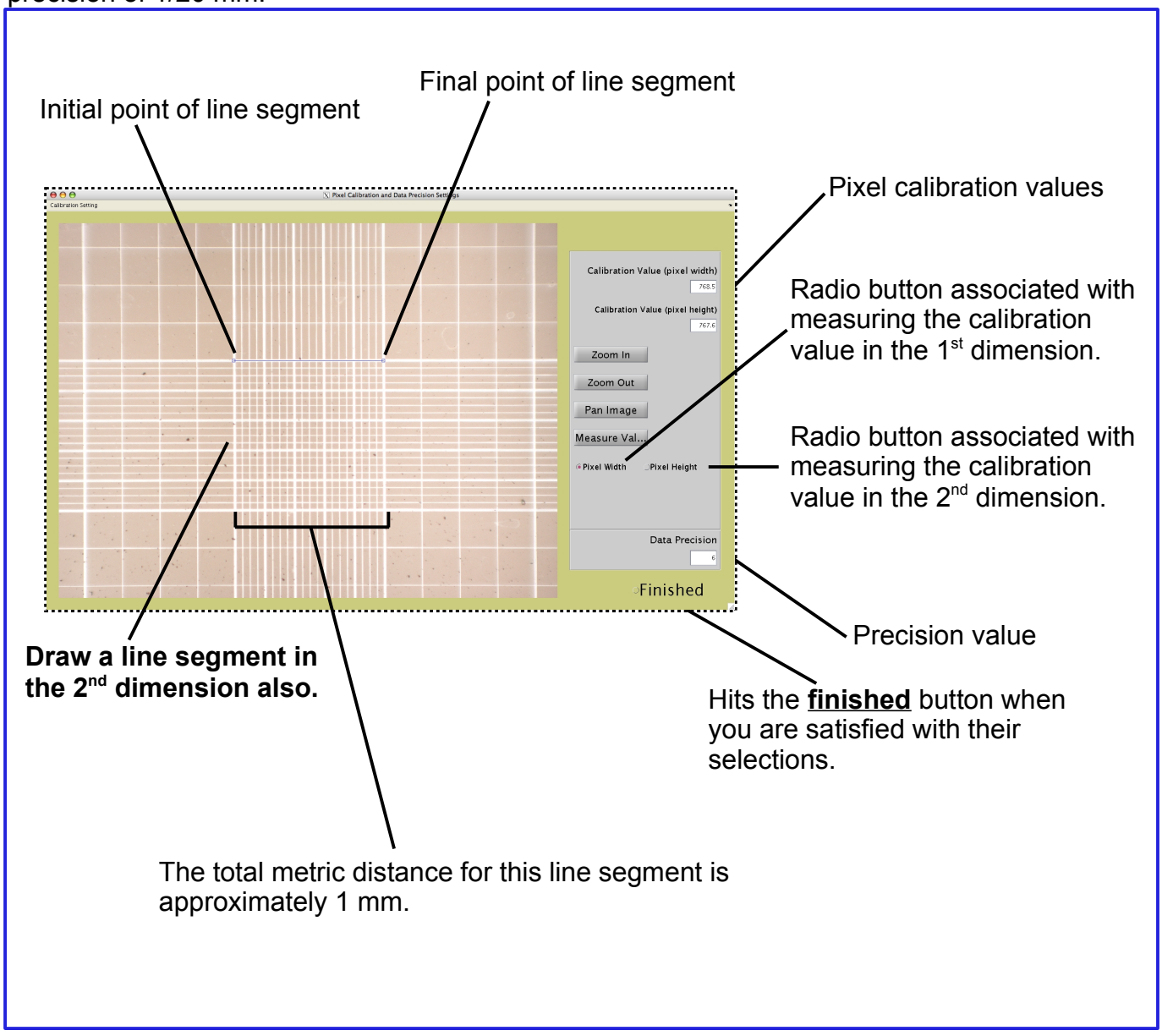
Calibration file prompt



Calibration Image

Step by Step Process continued

The calibration values for the current image directory are measured directly from the calibration image. The calibration image shown in the window below is a hemacytometer scale that was photographed under the same microscope (and settings) that took the root cross-section images. It is the important that microscope settings do not deviate significantly between the cross-section image and hemacytometer image. This variation would lead to measurement and process errors. The hemacytometer image in the GUI window below has a maximum precision of 1/20 mm.

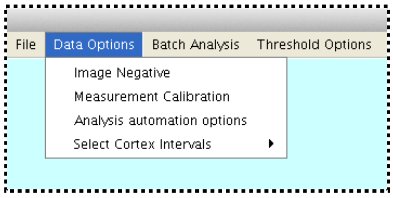


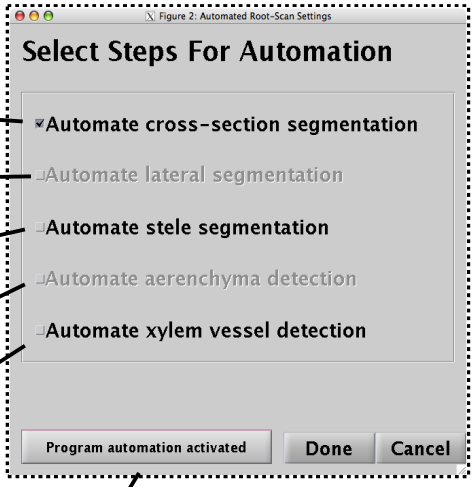
Automating Different Phases of the RootScan Analysis

There are five major steps performed on a single cross-section image: cross-section segmentation (pages 20-21), lateral root isolation, stele/cortex segmentation, lacunae selection and xylem vessel selection. Of the five steps, three can be fully automated by the user. Caution must be used when deciding to automate any RootScan step since the automation process could lead to measurement and process errors. The steps that are recommended for automation are enabled by default.

Setting the Automated Options from RootScan Main Menu Bar

The automated options window is accessed directly from the main RootScan window menu bar under 'Data Options:Automated Options' option. When this option is selected a separate window appears in the foreground of the main RootScan window, allowing users to interactively select which steps are automated.





Cross-section segmentation automation check box (enabled)

Lateral isolation automation check box (disabled)

Stele/cortex segmentation automation check box (enabled)

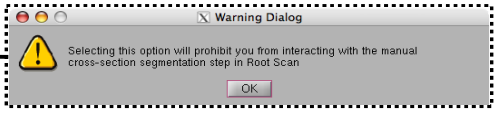
Aerenchyma selection automation check box (disabled)

Xylem vessel isolation automation check box (enable)

Program automation pushbutton toggles on/off when pressed letting the user know if the automated options are activated or not.

When each of the enabled checkboxes are checked a warning message appears in the foreground of the Automated RootScan Settings reminding the user of the consequences of this decision.

Warning dialog box for cross-section segmentation automated options



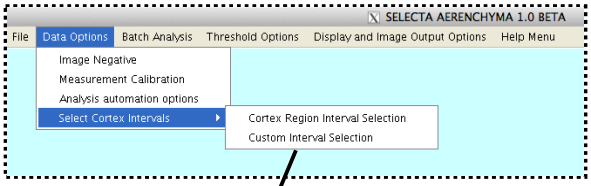
Warning dialog box for stele/cortex segmentation automated options

Warning dialog box for xylem vessel automated options

Cortex Interval Settings

Rootscan identifies objects in the cortical region and provides data on cortical cell number, cortical cell size and the number of files. In addition, cortical cell parameters can be calculated for radial zones within the cortex. The user has the ability to set the partition of each band at the locations suitable for the type of data desired. There are three standard settings and one custom setting that allow users to select the number and location of partitions. The default partition setting is the '4-Equal-radial Regions' setting. Users can experimentally determine which settings are appropriate for their work by testing each setting using the individual image options (See Selecting Image Files and Image Directories).

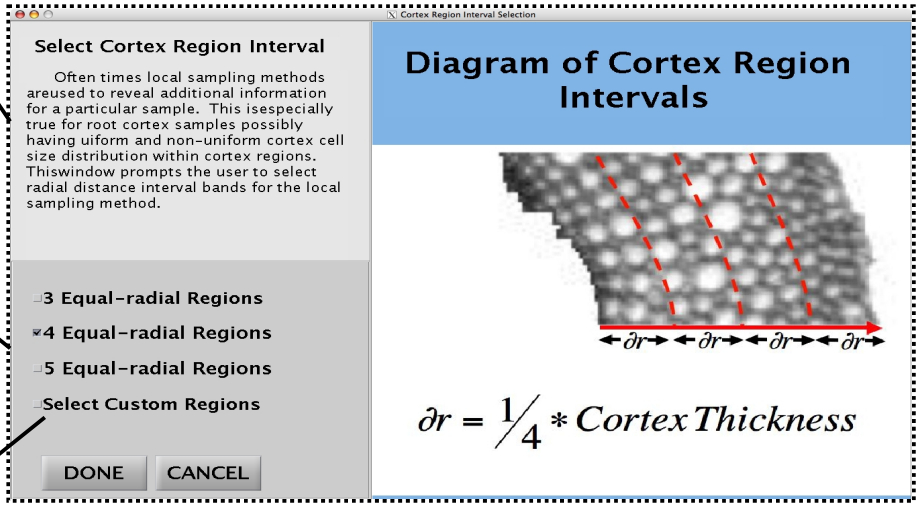
The cortex interval settings are accessed by 'Data Options:Select Cortex Interval:Cortex Region Interval Selection ' option. When this option is selected a separate window appears in the foreground of the main RootScan window, allowing users to select the appropriate cortex intervals.



To set your own customized cortex interval boundaries, select the 'Custom Interval Selection' option directly from the menu bar from the main RootScan window.

Information box

Check box panel for cortex interval partition selection. The diagram will reflect the number of intervals selected.



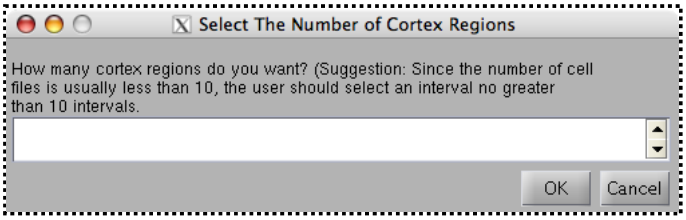
If you select "Select Custom Regions" option no diagram will appear. The option will be applied when you press the "Done" button.

Custom Cortex Interval Settings

Users can access the custom cortex interval settings GUI directly by selecting 'Data Options:Select Cortex Interval:Custom Cortex Intervals' option directly from the RootScan main menubar. Before this GUI window appears on the screen an input dialog box appears that allows users to type in the number of customized interval partitions desired. Once users type a valid number (i.e. non-negative integer) the dialog box terminates and the custom cortex interval settings GUI appears for user interaction.

Entering the Number of Cortex Interval Partitions

Users enter the number custom cortex intervals used for their interval partition placement. This input dialog box will not terminate until the user enters a valid response.

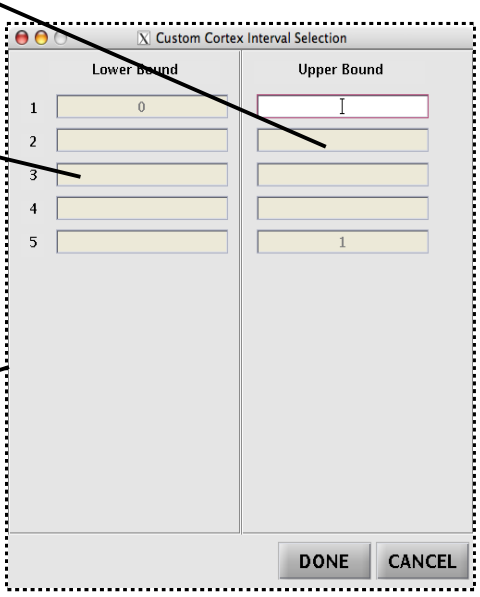


Positioning the Custom Cortex Interval Partitions

Define the partition locations in the upper bound column. Values must be real numbers between 0 and 1.

Lower partition boundaries are set automatically based on entries in the right-hand column.

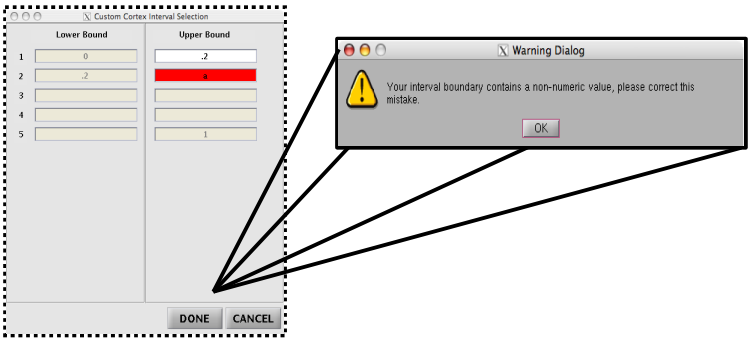
Custom cortex interval partition placement GUI



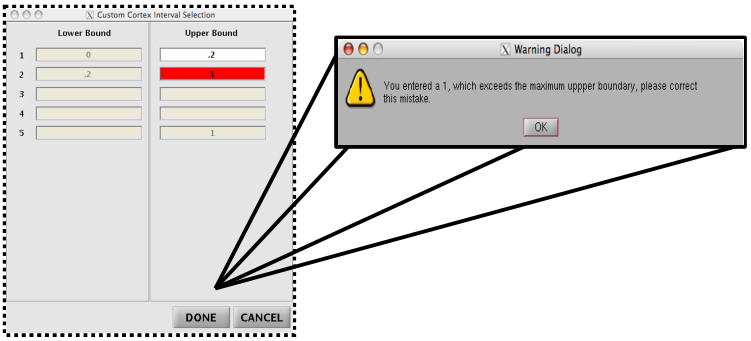
Correct and Incorrect Custom Cortex Interval Partition Entries

Examples of custom cortex values and the response by RootScan are shown below..

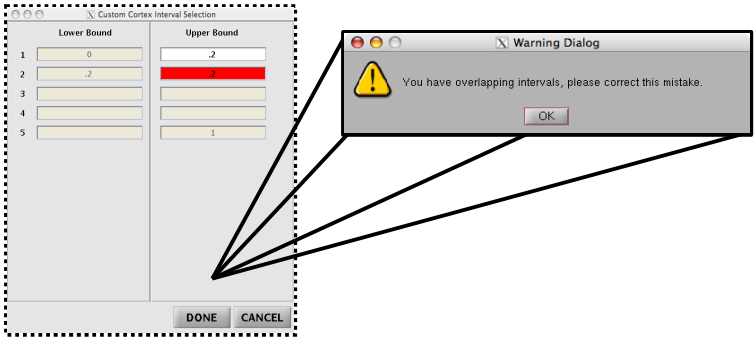
Incorrect Interval Entries



The GUI window to the left shows an incorrect entry of .2 and an 'a' in the first and second text boxes, respectively. The second entry is not valid and RootScan creates a message that the second entry is not valid by flagging the text box in red and issuing a warning.



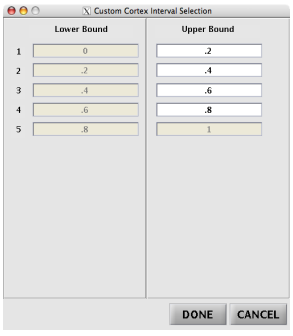
The GUI window to the left shows a .2 and a number 1 in the first and second text boxes, respectively. The second entry is not valid since the range of values must fall between [0,1]. RootScan informs the user of this case by flagging the text box red and issuing a warning.



The GUI window to the left shows a .2 and .2 in the first and second text boxes, respectively. The second entry is not valid since the two interval bands cannot have the same upper boundary. RootScan informs the user of this case by flagging the text box red and issuing a warning.

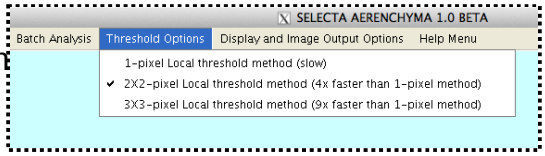
Sample Entry

The figure to the right snows a series of correct upper boundary entries made by the user. These entries can be in either the decimal or fractional form. These values along with other cortex interval data, are saved into a data cell array located in the RootScan programs directory. When the user is in the individual image mode this cell array is read into the session. When the user begins analyzing an entire directory of images in batch processing mode these settings are read into the session and saved in the image directory chosen by the user.



Localized Image Thresholding

Localized image thresholding is used when you want to increase image binarization accuracy at finer scales with varying contrast. RootScan's local binarization algorithm uses the pixel attributes of a sample window locally centered around the current pixel. Pixels are deemed foreground or background if they meet the specified requirements in defined in the algorithm.



The threshold options are set directly from the RootScan main menu bar.

Different Thresholding Methods

1 pixel method evaluates only one pixel per iteration. This produces good accuracy.

27 pixels converted in 27 iterations

1	2	3	4	5	6	7	8	9
10	11	12	13	14	15	16	17	18
19	20	21	22	23	24	25	26	27

2X2 pixel method evaluates four pixel per iteration. This results in a four-fold reduction in computation time. You lose some accuracy due to spatial bias but the results are still very reliable.

32 pixels converted in 8 iterations

1	1	2	2	3	3	4	4
1	1	2	2	3	3	4	4
5	5	6	6	7	7	8	8
5	5	6	6	7	7	8	8

3X3 pixel method evaluate nine pixel per iteration. This results in a nine-fold reduction in computation time. You lose more accuracy due to spatial bias.

27 pixels converted in 3 iterations

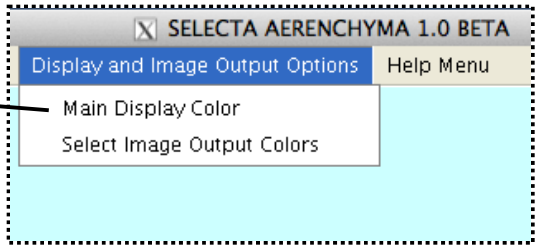
1	1	1	2	2	2	3	3	3
1	1	1	2	2	2	3	3	3
1	1	1	2	2	2	3	3	3

Setting the Background Color of the Main RootScan Window

Users have the option of changing the background color anytime during their session.

Set Background Color via Main Menu Bar

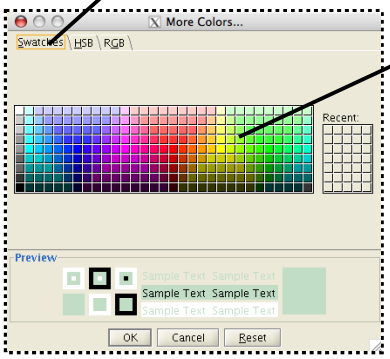
Users can change the RootScan background color by selecting 'Display and Image Output Options:Select Image Output Colors.



This option opens up a standard universal Matlab GUI window showing various colors and an additional color options pushbutton.

An additional GUI window, containing a series of color options, is shown when users select the 'More Colors...' pushbutton.

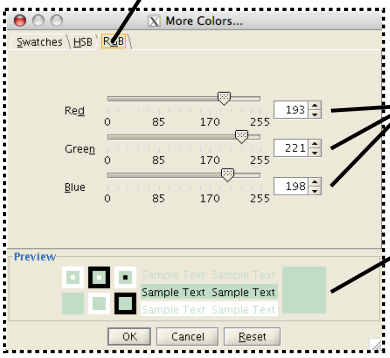
You can choose their colors using three selection modes: Swatches, HSB and RGB modes. Swatches option is currently shown.



Secondary GUI window

Under the swatches option, users are limited to the colors shown in the color grid.

The same secondary GUI window with the RGB option currently shown.

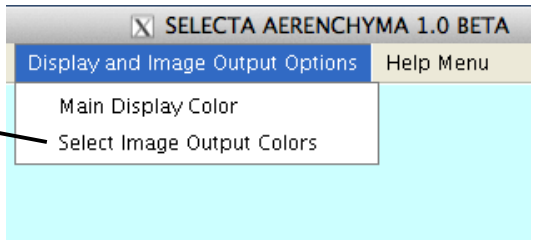


Use the slider to change the red, green and blue color channels

When any of the sliders are adjusted the color will change

Setting Color Preferences for the Reference Image and Highlighted image via Main Menu Bar

You can change the reference image and highlighted image color preferences by selecting 'Display and Image Output Options:Select Image Output Colors.



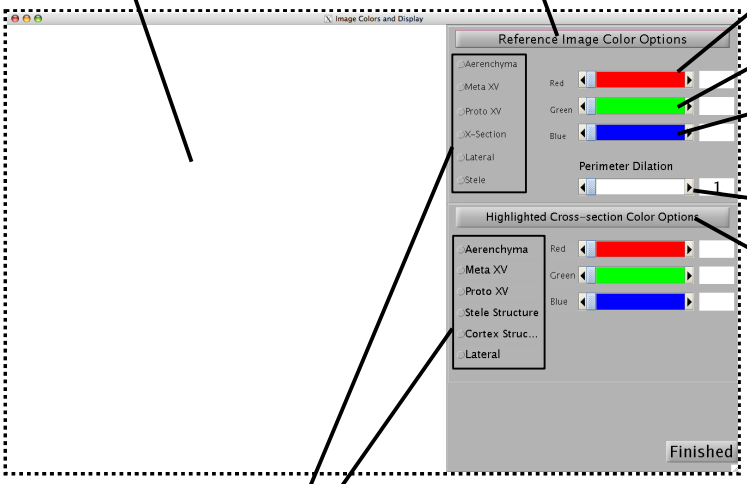
Select 'Reference Image Color Options' pushbutton to show the reference image with the current color preferences for the six reference regions for all RootScan analysis steps

The window is initialized without any image

Interactive color slider (Red color channel)

Interactive color slider (Green color channel)

Interactive color slider (Blue color channel)



Interactive Perimeter dilation slider

Select 'Highlight Cross-section Color Options' pushbutton to show the highlighted cross-section image with the current color preferences for the six anatomical regions of root cross-sections

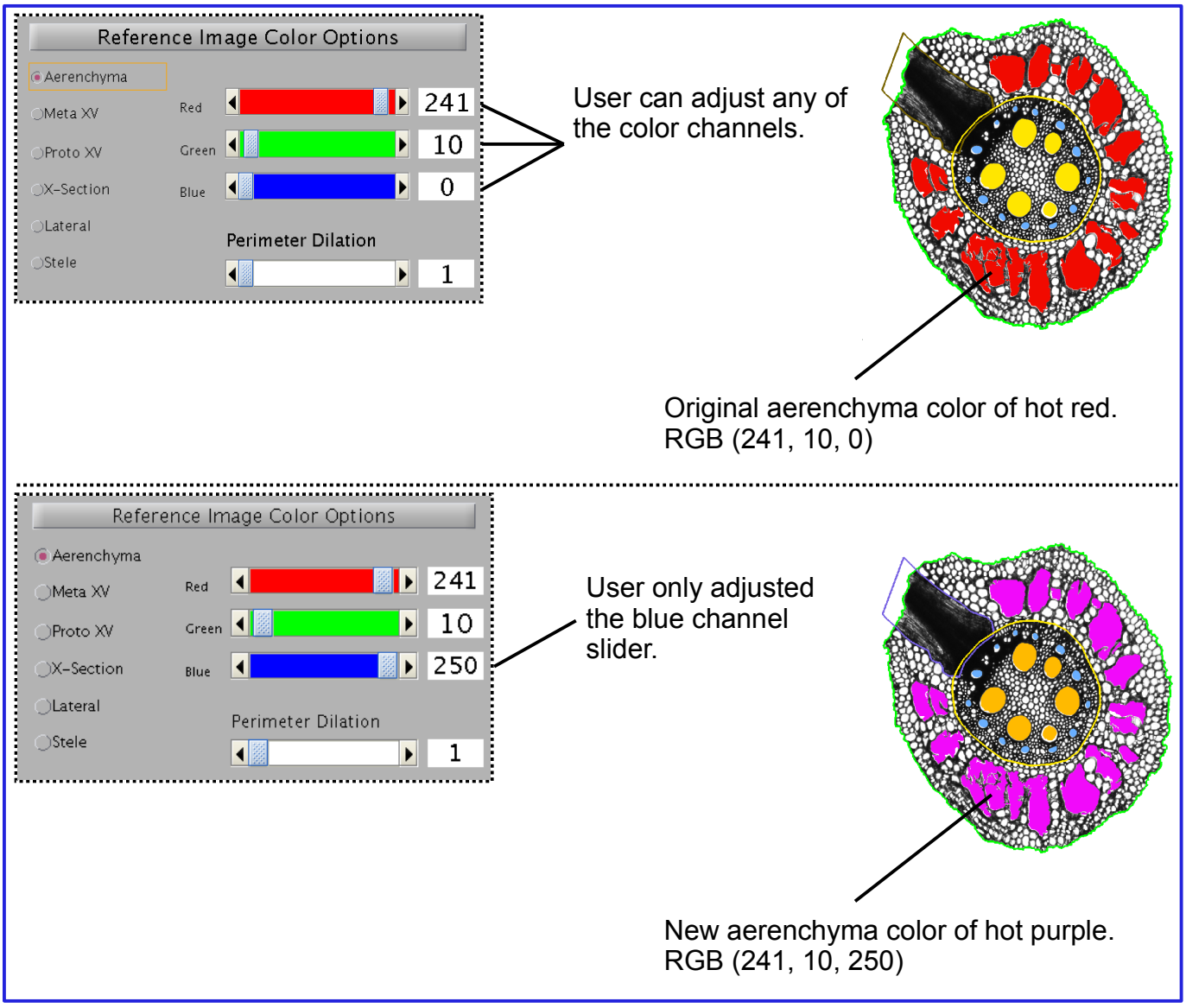
The radio buttons from each panel will activate the current color settings for that particular reference region in its parent panel. When the user changes the position of the red, green and blue color sliders, the current color settings will change.

Interactively Adjusting Cross-section Region Colors for the Reference Image Using Interactive Slider Bar

The reference image keeps track of all RootScan analysis steps by displaying the results in a color coded image. After the completion of each step, the reference image is augmented with the results of the most recent step. Once the analysis of each root cross-section image is completed, there are six possible color coded regions in the reference image. These six regions are the following: lacunae, metaxylem, protoxylem, x-section perimeter, lateral root perimeter and stele perimeter. This image is for display purposes only and is saved in the Image_Output directory contained in the parent image directory.

Adjusting Colors Using Slider Bars.

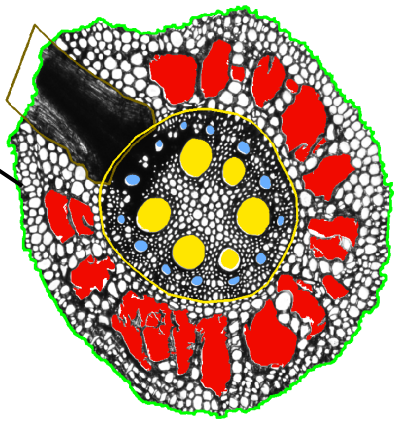
Three color bars for the red, green and blue color channels are provided so users can customize their reference image shown in the right-most region in the main RootScan window. The box below shows an example of a user changing the aerenchyma color.

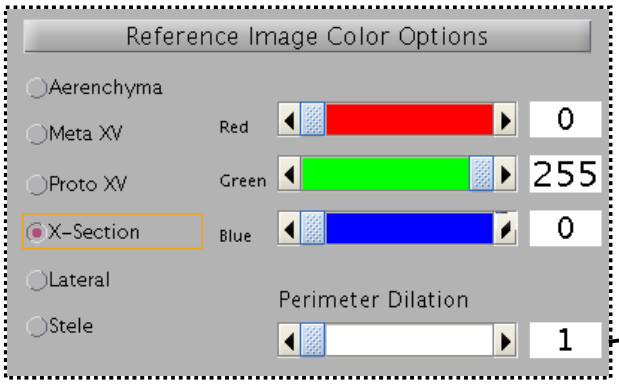


Adjusting Perimeter Dilation Preferences for Reference Image Using Interactive Slider Bar

Perimeter dilation preferences are only set for three regions: cross-section perimeter, lateral root perimeter and stele perimeter. When any of these three perimeter regions are selected, the user can adjust the color and perimeter dilation (i.e. boldness) by adjusting their respective slider bars. Below is an example of perimeter dilation changes made to the cross-section perimeter.

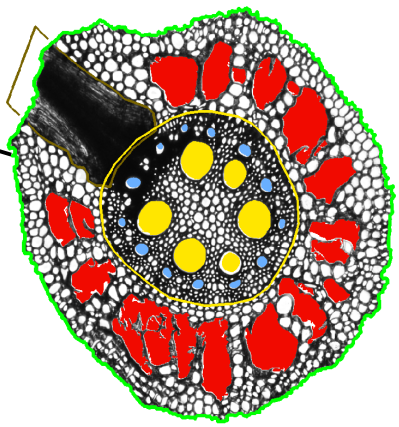
Original cross-section dilation of one pixel radius highlighted lime green

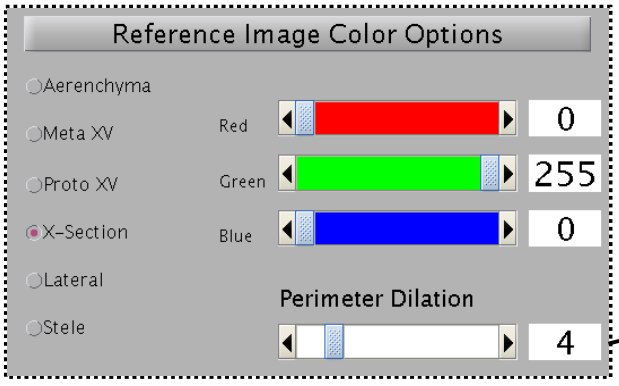




The initial radius of dilation is 1. The user can adjust this value.

Cross-section dilation changed from one pixel radius to four pixel radius units. The perimeter is 4 times as thick now. Highlighted color remains lime green





The user moved the slider until the radius of dilation equals 4.

Analyzing Cross-section Images in RootScan

RootScan Checklist Prior to Starting Batch Analysis

Check/measure calibration and precision settings

Calculate and verify their image calibration value and data precision settings prior to initializing batch analysis mode on any directory. **These settings remain static throughout the entire batch session . To change calibration and precision settings see pages 3-4.**

Check/verify automated options in Data Options

Verify which RootScan steps are automated during the batch processing mode. Keep in mind that you will not be able to interact with any step that is automated so the user is cautioned about implementing automation instructions for a given step. **These settings remain static throughout the entire batch session. To change automated options settings see page 5.**

Check/verify cortex interval settings

Test and verify their cortex interval settings prior to initializing batch analysis mode on any directory. You can validate cortex interval settings by testing a few images one at a time and adjusting the intervals until satisfied. **These settings remain static throughout the entire batch session. To change cortex interval settings see pages 6-8**.

Check/verify thresholding options

Verify which thresholding method is suitable for the current image directory. You can test individual images using the three thresholding options. **These settings remain static throughout the entire batch session. To change thresholding options see page 9.**

Check/verify color options

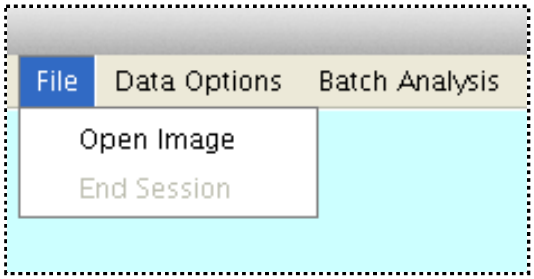
You have the option of defining cross-section color preferences for the reference image and highlighted cross-section image during any part of the batch processing mode since these images are used for display purposes only**. These settings, unlike the previous settings, can be changed at any time in batch processing. To change image color options see pages 10-14.**

Selecting Image Files and Image Directories

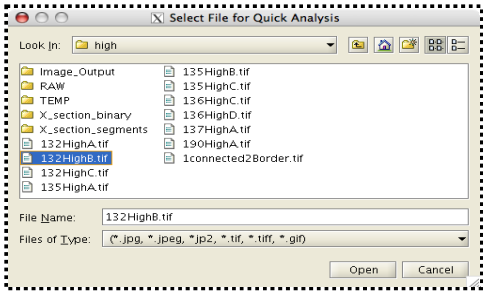
There are two analysis modes offered in RootScan: individual image analysis mode and batch processing mode. The individual image analysis mode allows you to test and verify which settings are appropriate for a given directory. Once these settings are determined you can batch process these image using the batch processing mode.

Opening individual image files

Users can start analyzing individual images by selecting the 'File:Open Image' option under the main menu bar on the RootScan graphical user interface (GUI). This selection will prompt a user to select one individual image file at a time.



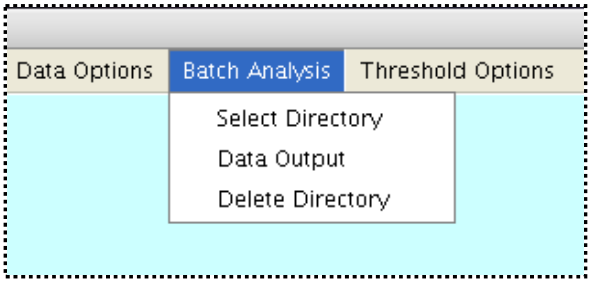
Selecting individual image file from menubar



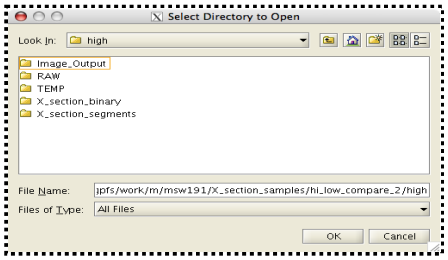
This window allows users to select one image within a directory.

Opening directories for batch analysis mode

Users can start analyzing entire directories for batch analysis mode by selecting the 'Batch Analysis:Select Directory' option under the main menu bar on the RootScan GUI. This selection will prompt the user to select an entire directory for batch processing mode.



Selecting batch processing mode options from menubar



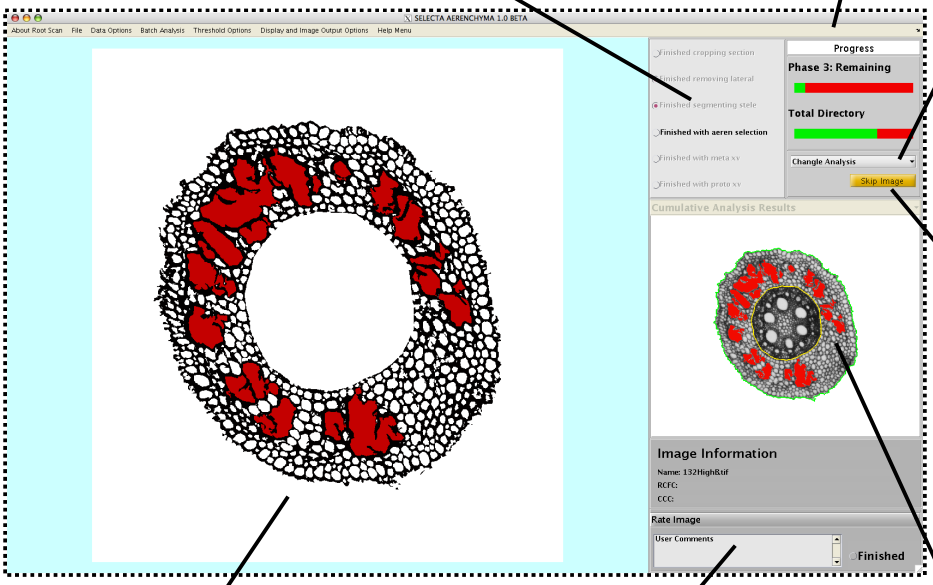
This window allows users to select an entire image directory for batch analysis mode.

Main RootScan Interface During Image Processing Mode

Finished Buttons Panel:
Select the radio button associated with the current step when you aresatisfied with their current selections.
Note: the radio button associated with that step is the only button enabled at that time.

Progress Bar Panel:
For each phase (i.e. cross-section segmentation, image binarization and general cross-section analysis steps) the status is shown to inform you of the progress through a phase and directory. Page 19 describes all three RootScan phases.

Change Analysis Button:
Selects the options on the dropdown box associated with the current interactive step that needs additional correction.



Skip Image Button:
You can skip an image when this button is pressed.

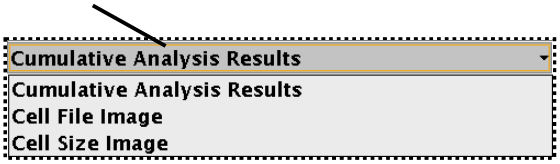
Main Image:
This is the area you will be interacting with images.

Reference image: The reference image is updated whenever the you make a change to cross-section components in the main image area. There are three reference images accessible to users. See page 18 for more information.

Image Information and Comments Panel:
This panel displays the image name, number of radial cell file count (RCFC) and number of cortical cells (CCC). These should be checked for accuracy. Once the you complete all interactive steps, you can rate the image by clicking the dropdown menu box labeled ***Rate Image*** and type your comments in the editable text box with initial string "**User Comments**".

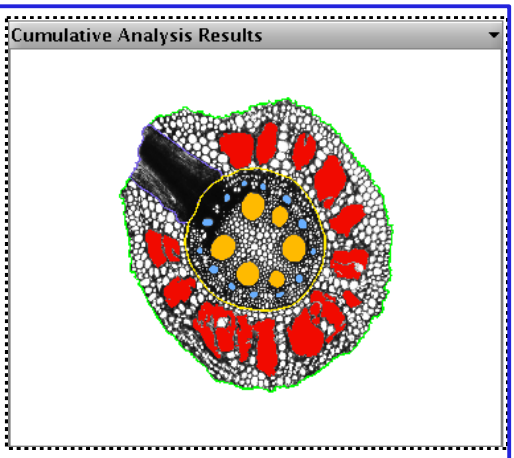
Reference Image Options

In addition to the standard reference image, alternative reference images (i.e. cortical cell file and cortical cell size distribution) are made visible when you select the **Cumulative Analysis Results drop down menu bar** above the current reference image. This drop down menu bar will only become active when you complete all steps for an image.



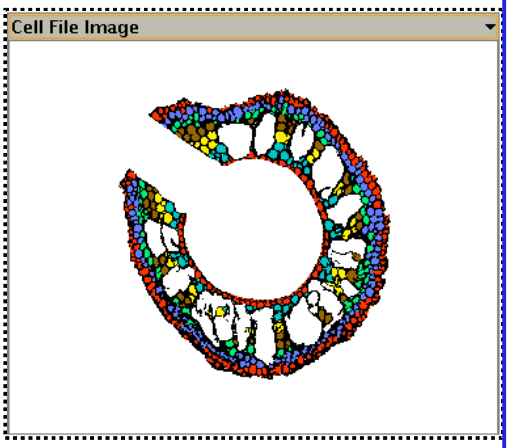
Reference Image

The reference image keeps track of the results from each RootScan analysis step by highlighting those target regions a particular color specified in the Display and Image Output Options menu bar tab in the main RootScan GUI window. Selections will appear in the reference image as they are made. This is the default image when the program proceeds to the next image in the current directory.



Cell File Image

The radial cell file image is an approximate highlighting scheme for all radial cell file bands as they are delineated in the cortex. This image is produced after the radial cell file count has been calculated in the lacunae selection process. The user displays this image by selecting the 'Cell File Image' option in the Cumulative Analysis Results drop down menu bar.



The Three Phases of Root Cross-section Analysis

Phase I: Root Cross-section Segmentation

You will review and modify the automatic cross-section result at this time. The resulting image will contain only the root cross-section. This image is sent to Phases II and III of the analysis. See pages 19-20 for complete description of this Phase.

Note: If you are performing a batch analysis you will perform this action on all raw root cross-section images before proceeding to Phases II and III.

Phase II: Cross-section Binarization

All image results from Phase I are passed to the cross-section binarization algorithm. The resulting image is the binary representation of the grayscale cross-section, with cell wall pixels coded black and non-cell wall pixels coded white. This phase is completely automatic, therefore no interaction is needed.

Note: If you are performing a batch analysis the computer will perform this action on all root cross-section images before proceeding to Phase III. Get up, stretch and go do something else until this phase is complete. This phase may take some time. See page 37 for more information about Phase II runtime statistics.

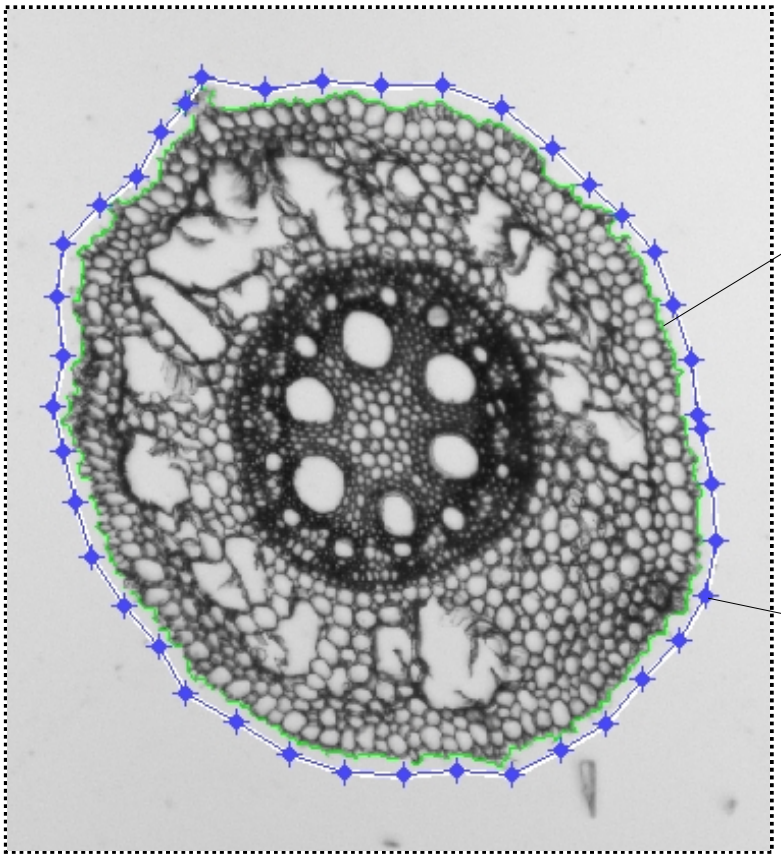
Phase III: Remaining Steps in Analysis

All image results from Phases I and II are passed to the remaining sequential analysis steps. During this phase you will complete the following steps for each image: Lateral Root Isolation (pages 21-23), Stele/Cortex Segmentation (page 24), Aerenchyma Selection (page 25), Metaxylem Vessel Selection (page 26) and Protoxylem Vessel detection (page 28). Each of these remaining steps includes automatic detection algorithms and its interactive counterpart.

Rootscan Phase I

Cross-section Segmentation Phase

The cross-section segmentation phase (Phase 1) consists of two steps. In the first step, automatic cross-section detection, the program defines the boundary of the cross section and displays the result. In the second step, manual cross-section selection, the user can adjust the boundary or create a new boundary. If the selection includes root hairs or external debris, they should be excluded by moving the blue polygon at any of the crosshair points. To draw a new polygon the user presses the 'n' key on the keyboard, which changes the pointer to a mini crosshair. The user can start drawing the new perimeter.



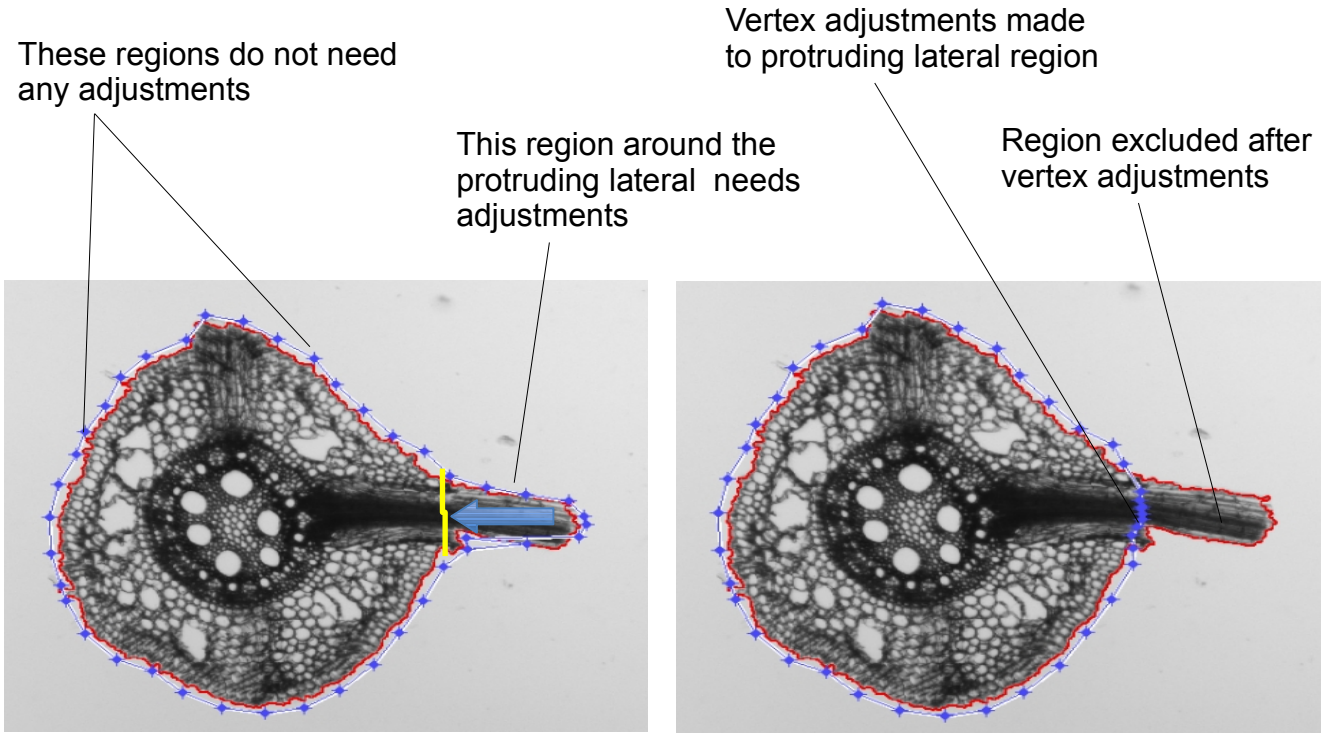
The result from the automatic cross-section detection process is represented by the green perimeter boundary. This boundary is not movable by user.

You can correct the automatic cross-section detection results by moving any of the blue polygon vertices. Pressing the 'x' key expands the polygon outward from the cross-section center. Pressing the 'c' key radially contracts the polygon toward the center.

Once you are finished with your selections, hit the '**Finished cropping section**' button.

Cross-Sections with Protruding Laterals

Sometimes users encounter cross-section images with protruding laterals or debris adjacent to laterals. In both cases the pixels contained in these region are not classified as cross-section pixels and should be eliminated. In this case, users need to pull all applicable vertices back toward the cross-section boundary should be reset using the interactive blue polygon. The bottom-left figure shows the stationary red cross-section border chosen by RootScan and initial interactive blue polygon both including a protruding lateral in the selection. The reason blue polygon perimeter name is prefaced by the words: 'initial' and 'interactive' is very important. The coordinates from the red cross-section border are used to form the initial interactive polygon. Notice how the blue polygon vertices coordinates are expanded radially outward from the red border. This was done so the user can view the results from the automatic cross-section segmentation step executed in RootScan. Correct the cross-section by dragging all applicable vertices back to the cross-section boundary. All vertices (i.e. blue dots) to the right of the yellow line need to be adjusted back to the yellow line. The bottom-right figure shows the result of performing such an action. There is another lateral lateral root in the example below, forming from the top of the image. Some users may adjust those vertices also since it could be considered protruding. **It is not necessary to adjust every vertex of the interactive polygon to correct the section boundary. Only inaccurate vertices need to be adjusted.** This is because the results from the user interaction are sent to another automatic cross-section segmentation step. The result reflects the combination of the secondary auto detection and any changes made by the user during the interactive portion of this phase.



Before user eliminates protruding lateral

After user eliminates protruding lateral

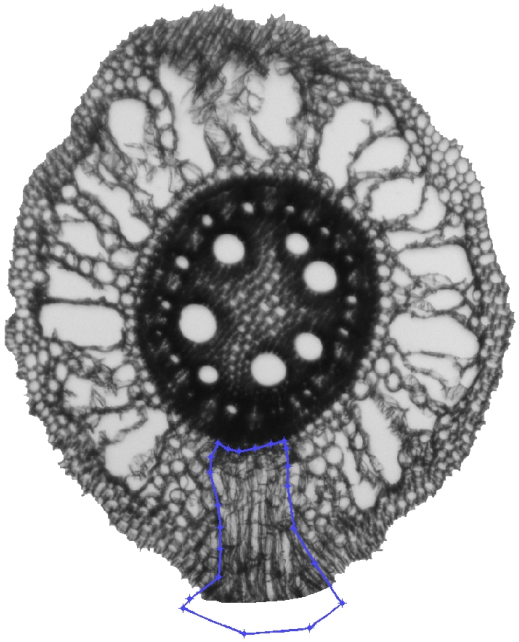
Rootscan Phase II

When you have completed Phase I, RootScan automatically transitions to the image binarization process. Since this process is entirely automatic you can work on another project until this phase is complete.

Rootscan Phase III

Lateral Root Isolation

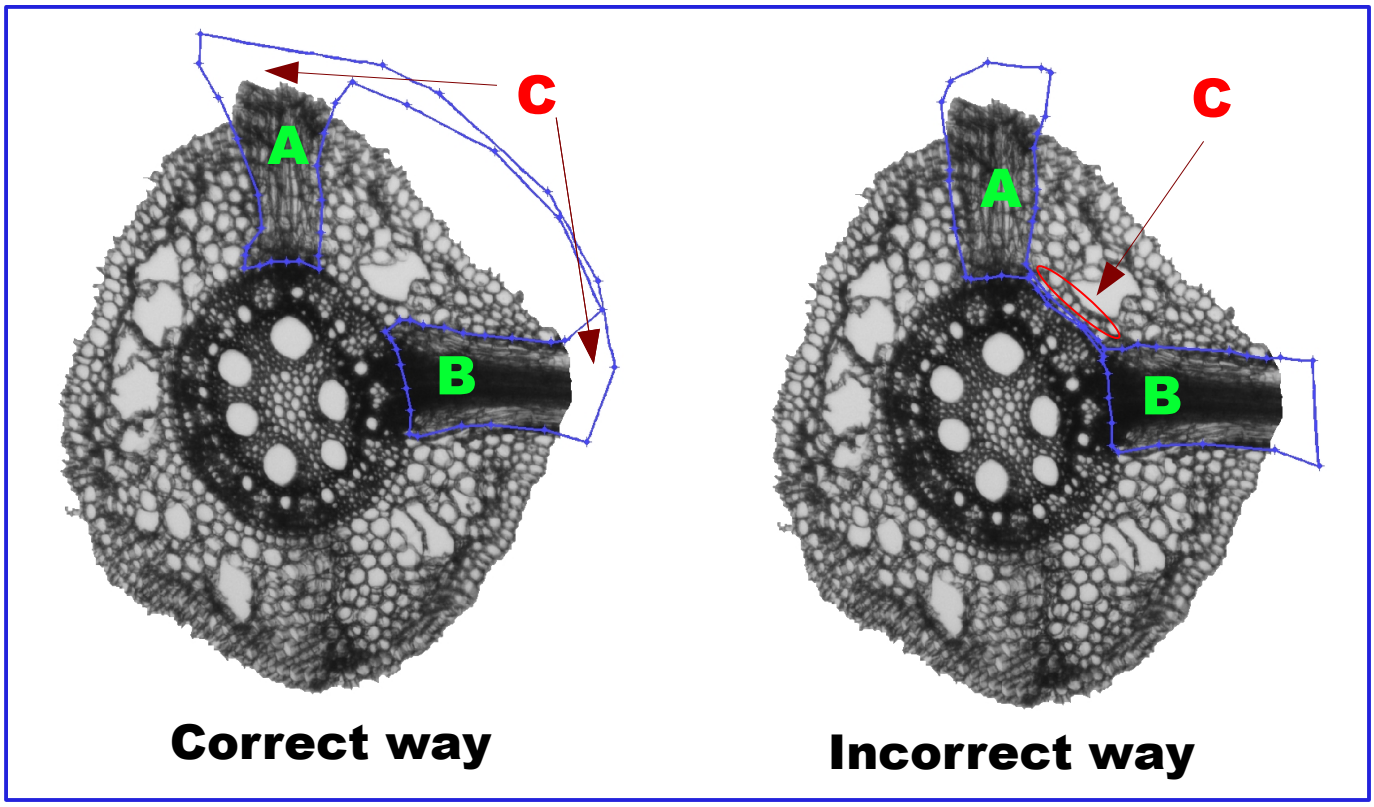
At this time there is no automated step for detection and exclusion of lateral roots in cortical regions. If there are 'N' of lateral roots, the user must eliminate 'N' number of lateral roots from the cortical region. Some cross-sections can accommodate up to 3-4 lateral roots, although this case is very rare.



The cross-section image to the left shows an image undergoing lateral root exclusion. You can start drawing the interactive polygon by placing your first vertex on any location on the lateral root border by a single click. Release the button and move the mouse to the next point. You should see a blue segment growing and following the data cursor. Once the data cursor is over the next possible vertex location the you can click again. Continue clicking around any lateral root(s) until you return to your starting point. You can close the polygon by double clicking or clicking on the first vertex drawn. Once the polygon is closed you can **drag** the vertices or **move** the entire polygon if mistakes were made during the initial placement.

Once you are finished with your selections, hit the '**Finished removing lateral**' button.

Correct and Incorrect way of Isolating Two Lateral Roots



The top-left image shows the **correct way** to isolate two lateral roots from cortical regions. Since the background is eliminated during cross-section calculation it does not matter where you draw outside cross-section images (i.e. the regions enclosed by C). The only thing that matters during this step is where you place your vertices in the cross-section.

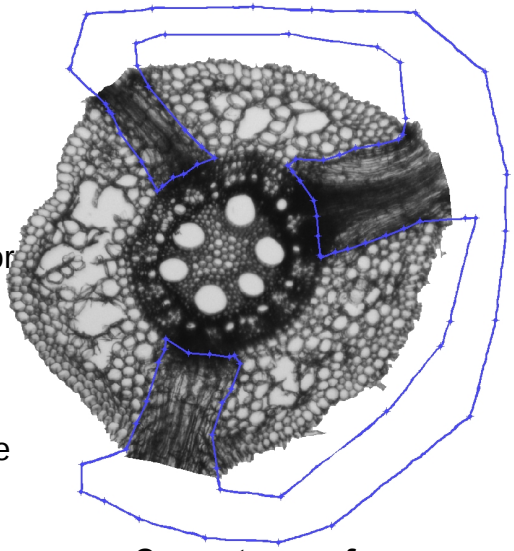
The top-right image shows the **incorrect way** to isolate two lateral roots from cortical regions. Region A and B are correct since the area enclosed by the polygon (excluding the background of coarse) constitute lateral root area. Region C is included in the lateral root area calculation, however, this region either belongs to the stele or extra-stele region. Therefore, drawing the interactive polygon in such a way would introduce measurement error in subsequent steps.

It is essential to correctly eliminate lateral roots from cortical regions so they do not compromise the quality of data derived from subsequent steps.

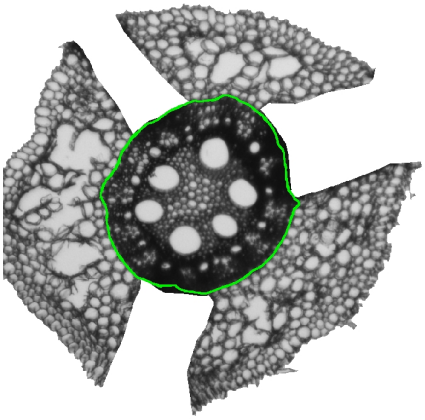
Correct and Incorrect Way of Isolating Three Lateral Roots

Correct way

The first image shows the correct way to isolate three lateral roots resulting in the correct lateral area estimation. Simply trace a path similar to that of images with 2 lateral roots. Once vertices are placed for two laterals they must transverse the background area in the opposite direction to isolate the third and final lateral region. The correct result is shown to the right.



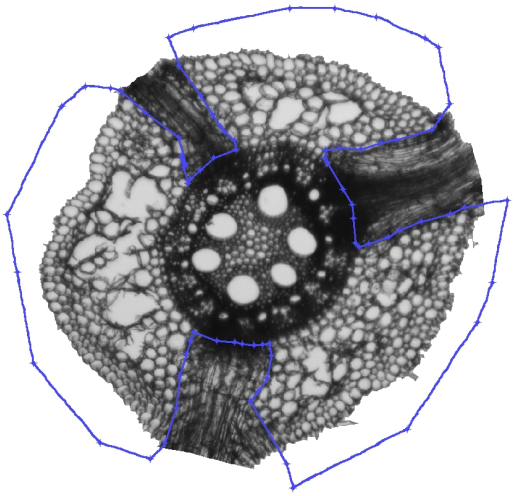
Correct way of drawing vertices



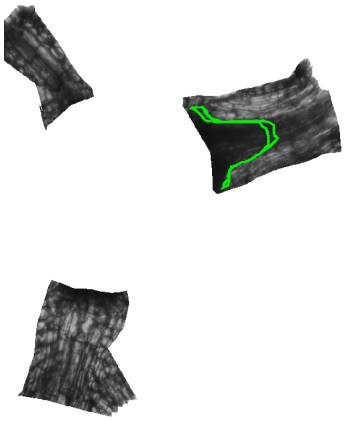
Correct Result

Incorrect way

The first image shows the incorrect way to isolate three laterals resulting in the total elimination of cross-section. Areas within the interactive polygon will be eliminated. If the polygon is drawn to include, rather than exclude, those lateral root regions are passed to the subsequent steps, thus making the rest of the analysis wrong. The incorrect result is shown to the right.



Incorrect way of drawing vertices

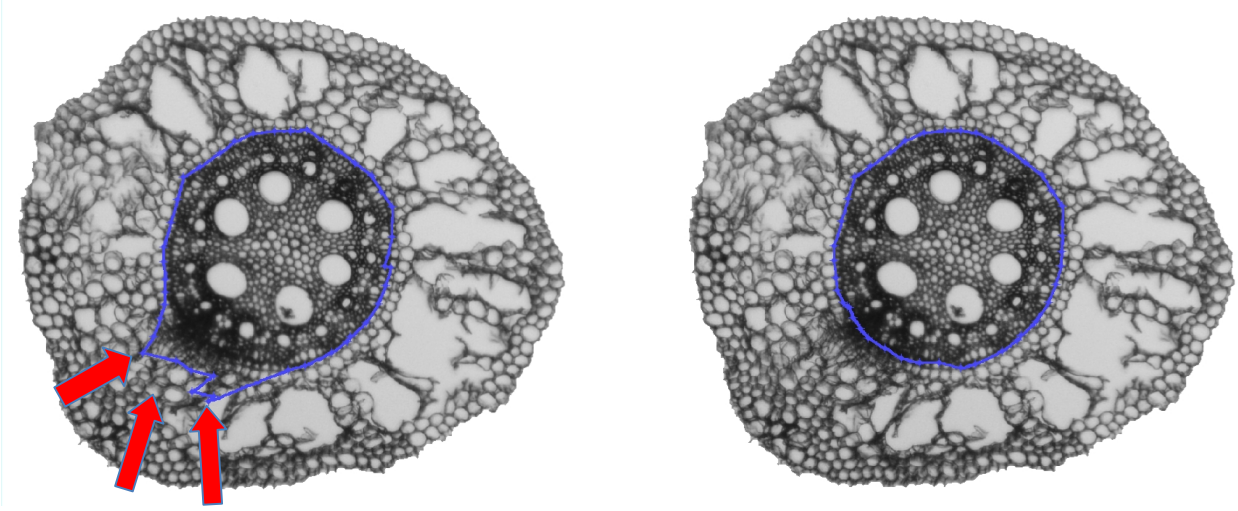


Incorrect Result

Stele segmentation step

Rootscan has an automatic stele segmentation step built in to reduce the amount of user interaction during this step. Once the automatic stele detection algorithm is completed you have the opportunity to correct the results. The image to the right shows the initial interactive blue polygon on the initial stele perimeter as define by RootScan. You can make adjustments to this interactive polygon as you see fit.

Usually the automated stele detection algorithm yields good results, so no adjustments are needed. However if the stele border is not correctly defined, you can adjust it by dragging the vertices of the blue polygon to the correct border in those areas where the border is not accurate. Below is an image needing corrections to the stele/cortex perimeter The stele perimeter in the bottom-left image is in error (see red arrows) and needs correction. Correct these vertices by dragging them to the proper locations (see bottom-right image).



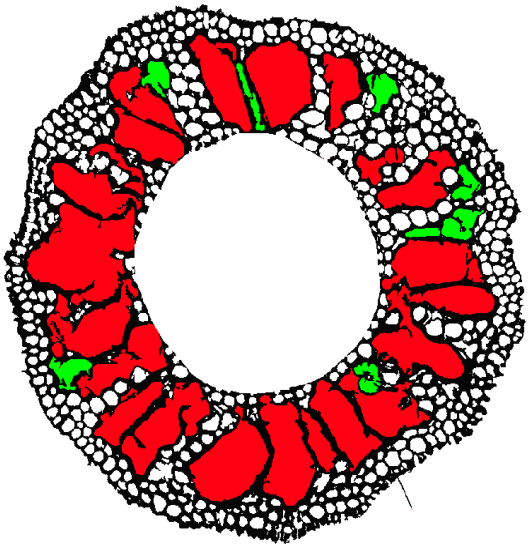
Before **After**

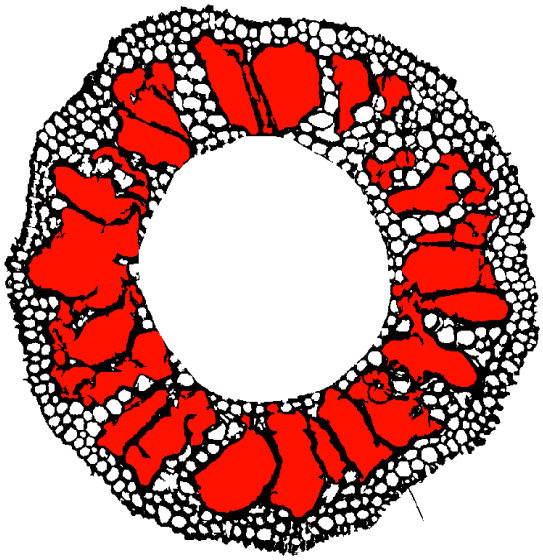
Once you have finished with your selections, hit the '**Finished segmenting stele**' button.

Lacunae Selection Process

Rootscan has an automatic aerenchyma detection algorithm built into it. The aerenchyma lacunae detection algorithm works by selecting objects based on their location and size relative to other cortical areas. Misclassified areas can be selected or deselected using the cursor.

In the bottom-left image, objects colored green were not identified as aerenchyma lacunae by RootScan, and were selected manually. Also, any objects misclassified as lacunae may also be deselected at this time. The result from the lacunae selection/deselection process are shown in the bottom-right image.



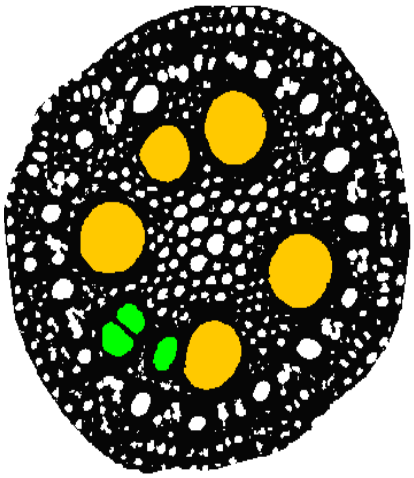


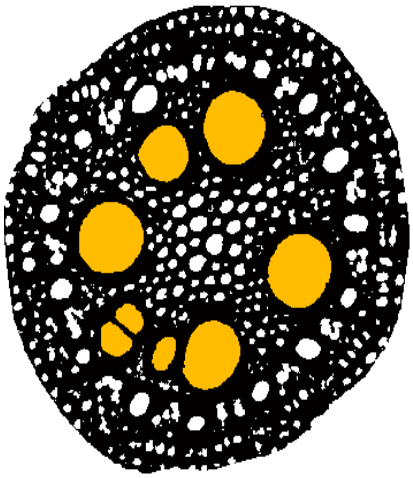
Note: The green lacunae in top-left image are not given this highlighted color in the Rootscan GUI. This is just for illustration.

Press the '**Finished with aeren selection**' radio button when you are finished with lacunae selections.

MetaXylem Vessel Selection Process

Rootscan has a automatic metaxylem vessel (meta XV) detection algorithm built into it. The automatic metaxylem vessel detection algorithm in RootScan is based on the size of objects in the stele. In the example to the left, RootScan has correctly highlighted the larger metaxylem vessels (yellow) but has missed several small ones (green). The undetected or misclassified areas can be corrected by clicking on the object to select or deselect . The result from the meta XV selection/deselection process are shown in the bottom-right image.



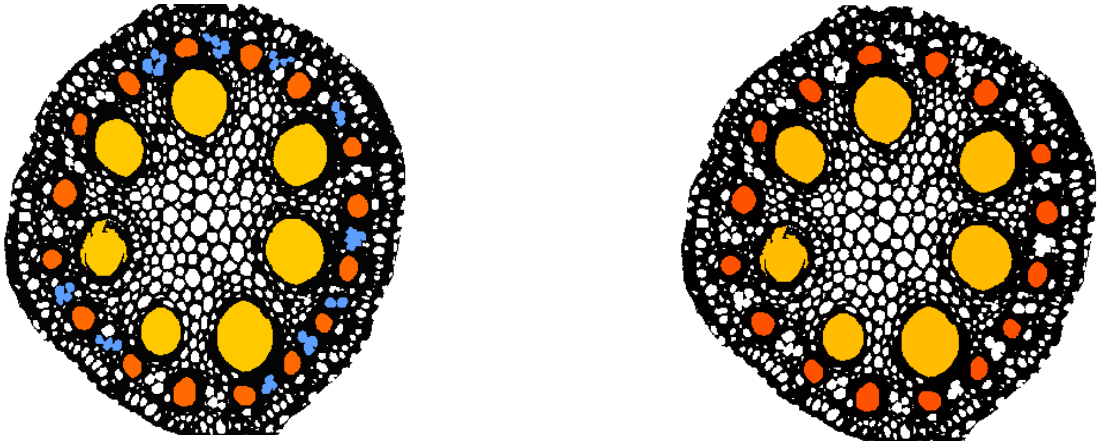


Note: The green meta XV left image are not given this highlighted color in the Rootscan GUI. This is just for illustration.

Press the '**Finished with meta xv**' radio button when you are finished with meta XV selections.

ProtoXylem Detection and Selection Process

Rootscan has a automatic protoxylem vessel (XV) detection algorithm built into it that is dependent on the meta XV selection step. The algorithm is based on relative size and location. Many of the proto XV areas are detected but some areas are misclassified as proto XV since these regions often have lower image contrast. The undetected or misclassified areas need user attention. In the example to the left, RootScan has correctly highlighted the larger proto XV (dark orange) but has missed several small ones (blue). The result from the proto XV selection/deselection process are shown in the bottom-right image.

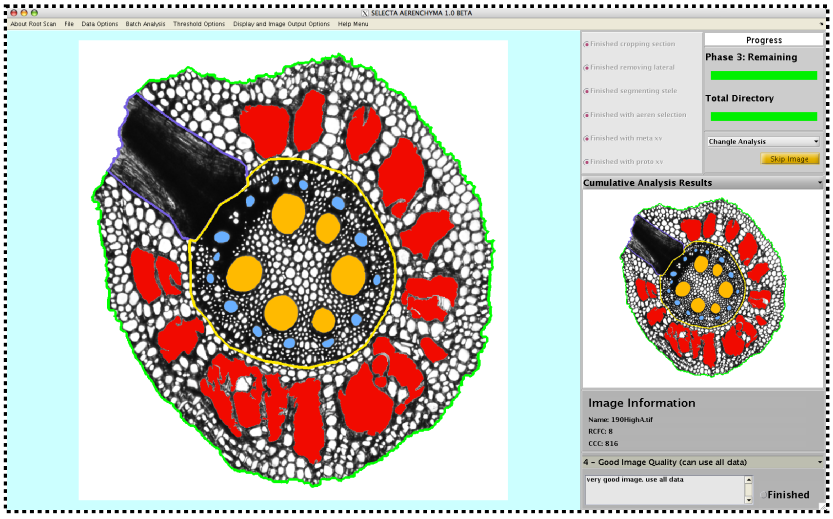


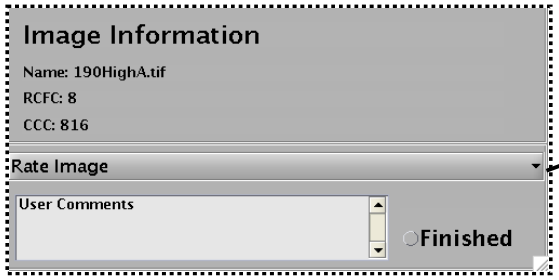
Note: The blue proto XV in top-left image are not given this highlighted color in the Rootscan GUI. This is just for illustration.

Press the '**Finished with proto xv**' radio button when you are finished with proto XV selections.

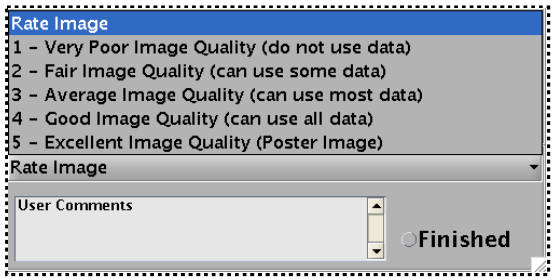
Image Quality and User Comments

Once you completed all steps you can reanalyze any part of the analysis or rate and make comments on your image.

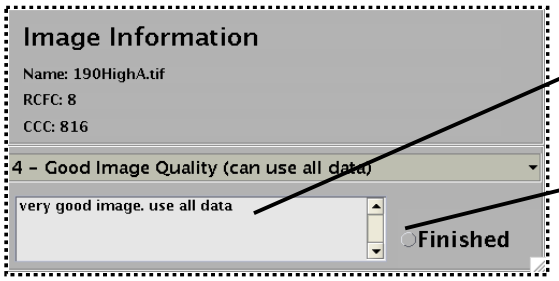




Select Rate Image dropdown bar give your image a numerical image quality rating.



Select one of five image quality indices.



Type your comments in this box. These comments will be printed to file as seen.

Select the Finished button when completed with all steps and quality ratings for each image.

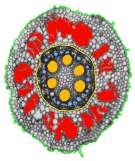
The Rootscan Image Directory Contents

Data Files Contained in an Image Directory

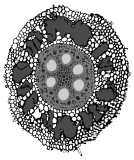
Image Files

Image Output Files

Once an image is completely analyzed, the reference image, highlighted cross-section image and radial cell file image are written to a subdirectory called 'Image_Output'.



Reference Image

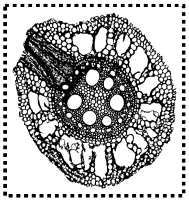


Highlighted Image

Radial Cell File Image

Cross-section Binary Images

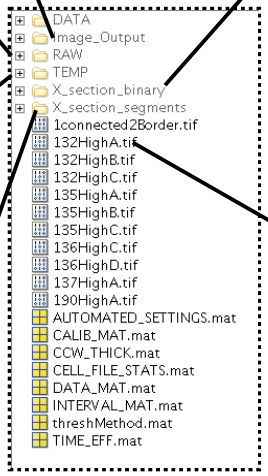
This subdirectory contains all cross-section binary images produced in Phase 2.



Binary Image

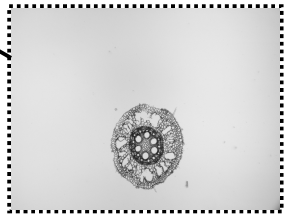
Raw RGB Images (not shown)

Prior to starting an analysis on a particular image directory, RootScan checks all images to verify that they are grayscale images. If the images are RGB images, RootScan defines a subdirectory called 'RAW', places all RGB image in that directory and converts all RGB images found in the parent directory to grayscale.



Temporary Images (not shown)

This subdirectory contains temporary images and matrix files that are loaded and updated during different steps of RootScan.

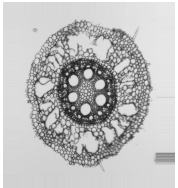


Original cross-section image

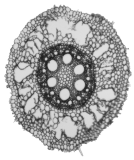
Phase 1 Cross-section Images

This subdirectory contains all truncated cross-section images produced in Phase 1.

Solid cross-section image



Cross-section buffer Image



Cross-section Image

Data Files Contained in an Image Directory, Continued

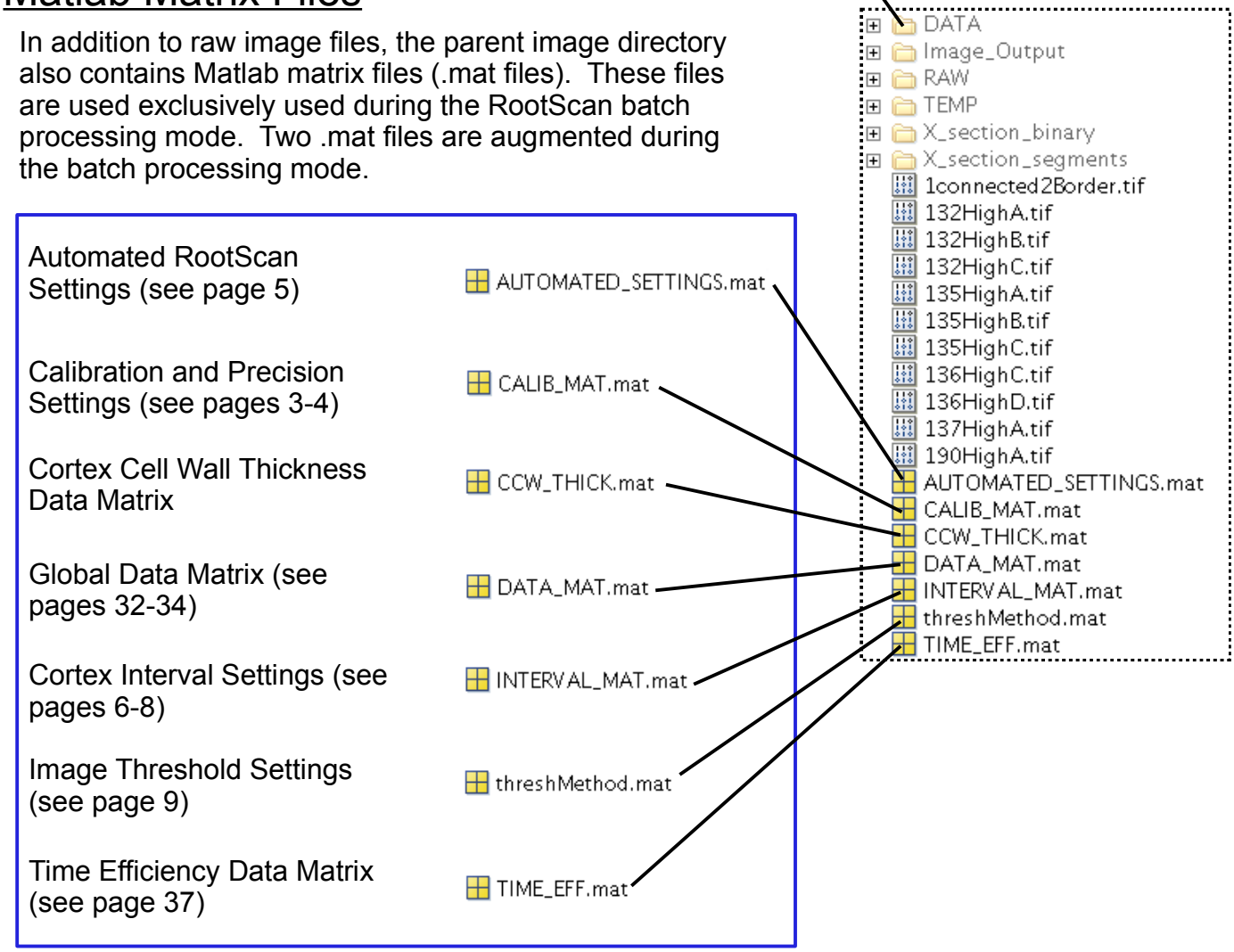
Data Output File

When a directory is completed, the data text file is saved in the subdirectory called 'DATA' in the current parent image directory. This is an ASCII file in 'tab' delimited format.

Filename	Calib Value/Threshold Method	XSCWA (mm^2)	RXSA (mm^2)	RXS_DIAMETER (mm)	CCWA (mm^2)	TCA (
132HighA.tif	Image Cal Value = 773 - Threshold Method = 2	0.331214	0.715710	0.958697	0.232151	0.55
132HighB.tif	Image Cal Value = 773 - Threshold Method = 2	0.348273	0.669618	0.927226	0.236944	0.50
132HighC.tif	Image Cal Value = 773 - Threshold Method = 2	0.338932	0.712736	0.956459	0.241704	0.54
135HighA.tif	Image Cal Value = 773 - Threshold Method = 2	0.419321	0.794515	1.008563	0.239252	0.55
135HighB.tif	Image Cal Value = 773 - Threshold Method = 2	0.389195	0.798011	1.010528	0.226226	0.56
135HighC.tif	Image Cal Value = 773 - Threshold Method = 2	0.387799	0.800870	1.017338	0.223820	0.56
136HighC.tif	Image Cal Value = 773 - Threshold Method = 2	0.559072	1.068133	1.168690	0.364126	0.80
136HighD.tif	Image Cal Value = 773 - Threshold Method = 2	0.492889	1.037900	1.153377	0.309496	0.78
137HighA.tif	Image Cal Value = 773 - Threshold Method = 2	0.408070	0.774164	0.996197	0.277659	0.58
190HighA.tif	Image Cal Value = 773 - Threshold Method = 2	0.383370	0.750401	0.982618	0.225420	0.51
1connected2Border.tif	Image Cal Value = 773 - Threshold Method = 2	0.425750	0.831305	1.033179	0.290223	

Matlab Matrix Files

In addition to raw image files, the parent image directory also contains Matlab matrix files (.mat files). These files are used exclusively used during the RootScan batch processing mode. Two .mat files are augmented during the batch processing mode.



The Rootscan Data File

Data Columns

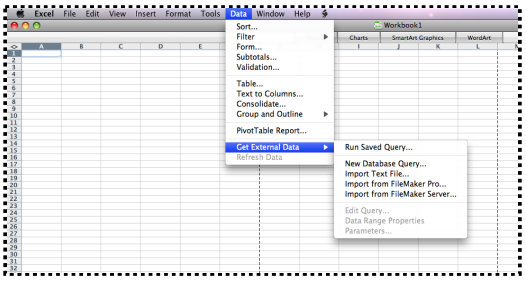
Column	Variable Name	Description	Metric Units
1/A	Filename	Name of image file	N/A
2/B	Calibration Value – Threshold Method	Ratio used to convert pixel distance to metric distance – image binary conversion method	$pixels/mm$
3/C	Root cross-section cell wall area	Total area of all cell wall	mm^2
4/D	Root cross-section area	Total area of all cross-section	mm^2
5/E	Mean root cross-section diameter	Mean cross-section diameter that considers root cross-section asymmetry.	mm
6/F	Cortical cell wall area	Total area of cortical cell wall	mm^2
7/G	Total cortical area	Total area of cortical region	mm^2
8/H	Mean cortical thickness	Mean radial distance between the inner and out cortical boundaries	mm
9/I	Stele cell wall area	Total area of stele cell wall	mm^2
10/J	Total stele area	Total area of stele region	mm^2
11/K	Mean stele diameter	Mean stele diameter that considers root cross-section asymmetry	mm
12/L	#Lacunae	Total number of aerenchyma objects in cortical region	Count
13/M	Median lacunae area	Media area of all lacunae objects	mm^2
14/N	Std. Dev. Of lacunae area	Standard deviation in area for all lacunae objects	mm^2
15/O	Smallest lacunae	Smallest aerenchyma object	mm^2
16/P	Largest lacunae	Largest aerenchyma object	mm^2
17/Q	Total aerenchyma area	Summation of all lacunae object areas.	mm^2
18/R	Cortical cell count	Total number of non-aerenchyma objects minus noise	Count

19/SS	Cortical cell area	Total area of all non-aerenchyma objects minus noise	mm^2
20/T	Radial cell file count	Total number of radial cortical growth rings	Count
21/U	# Metaxylem vessels	Total number of metaxylem vessels in stele	Count
22/V	Median metaxylem vessel area	Median area of all metaxylem vessel objects	mm^2
23/W	Std. dev.of metaxylem vessel area	Standard deviation of all metaxylem vessel object areas	mm^2
24/X	Smallest metaxylem vessel area	Smallest metaxylem vessel object area	mm^2
25/Y	Largest metaxylem vessel area	Largest metaxylem vessel object area	mm^2
26/Z	Median metaxylem vessel diameter	Median diameter of all metaxylem vessel objects	mm
27/AA	Std. dev. of metaxylem vessel diameter	Standard deviation of all metaxylem vessel diameters.	mm
28/AB	Smallest metaxylem diameter	Smallest diameter of all metaxylem vessel objects	mm
29/AC	Largest metaxylem diameter	Largest diameter of all metaxylem vessel objects	mm
30/AD	Total metaxylem area	Sum of all metaxylem object areas	mm^2
31/AE	Water conductance of metaxylem	Water flux parameter (metaxylem vessels)	m^4
32/AF	# Protoxylem vessel	Total number of protoxylem vessels in stele region	count
33/AG	Median protoxylem vessel area	Median area of all protoxylem vessel objects	mm^2
34/AH	Std. dev. of protoxylem vessel area	Standard deviation in area for all protoxylem objects	mm^2

35/AI	Smallest protoxylem vessel area	Smallest protoxlyem vessel object area	mm^2
36/AJ	Largest protoxylem vessel	Largest protoxylem vessel object area	mm^2
37/AK	Median protoxylem vessel diameter	Median diameter of all protoxylem vessel objects	mm
38/AL	Std. dev. Of protoxylem vessel diameter	Standard deviation in diameters for all protoxylem vessel objects	mm
39/AM	Smallest protoxylem vessel diameter	Smallest protoxylem vessel object by diameter	mm
40/AN	Largest protoxylem diameter	Largest protoxylem vessel object by diameter	mm
41/AO	Total protoxylem area	Sum of all protoxylem object areas	mm^2
42/AP	Water conductance (proto xylem)	Water flux parameter (protoxylem vessels)	m^4
43/AQ	Lateral area	Total area of all lateral root regions	mm^2
44/AR	Median cortex cell wall thickness	Median of all cortex cell wall thickness segments excluding all noise	mm
45/AS	Std. dev. In cortex cell wall thickness	Variation in the cortical cell wall thickness excluding all noise	mm
46/AT	Cortex cell wall thickness range	Range in cortical cell wall thicknesses excluding all noise	mm
47/AU ….N/Ak Where k is the # of bins	Median cell size (bin # j)	Median cortical cell size between radial distance bounds for bin # j	mm^2
47+k/Ak	Image rating	The integer rating users give images based on their overall image and data quality.	Integer
47+ (k+1)/A(k +1)	User comments	User types their custom comments	String

Opening Data Files in Microsoft Excel

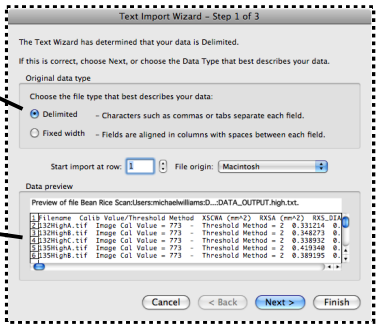
Open RootScan data file in Microsoft Excel by selecting 'Data : Get External Data : Import Text File...' in the Excel main menu bar.



Data import Step 1

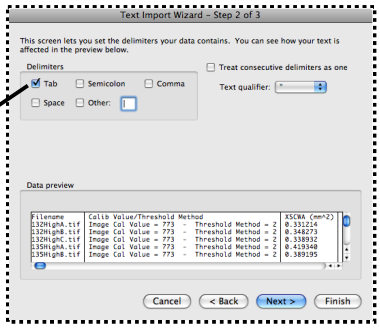
Make sure you choose the delimited option

Raw text data



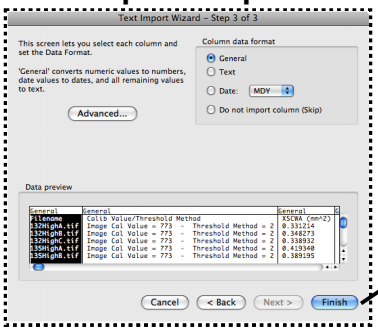
Data import Step 2

Make sure you choose the tab delimiter option

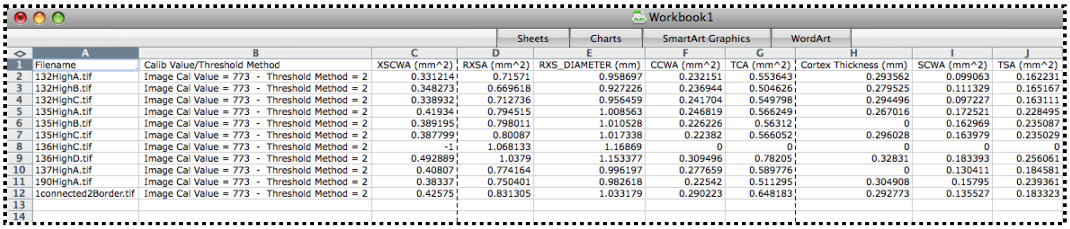


Data import Step 3

Click finished when done



RootScan data shown in Microsoft Excel

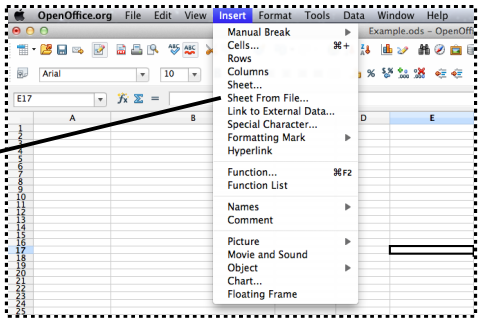


Workbook1

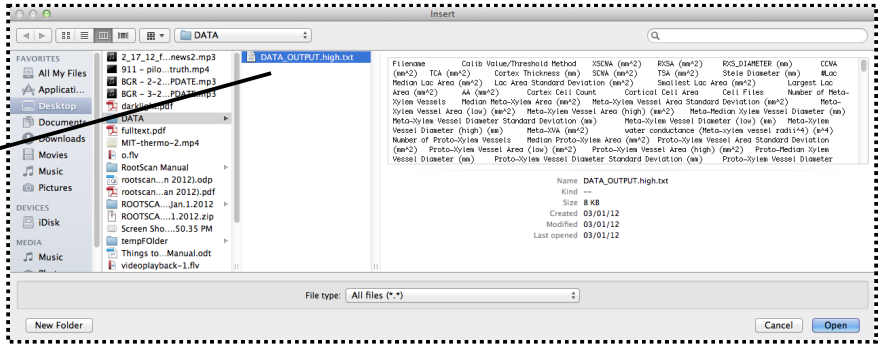
	A	B	C	D	E	F	G	H	I	J
1	Filename	Calib Value/Threshold Method	XSCWA (mm^2)	RXSA (mm^2)	RXS_DIAMETER (mm)	CCWA (mm^2)	TCA (mm^2)	Cortex Thickness (mm)	SCWA (mm^2)	TSA (mm^2)
2	132HighA.tif	Image Cal Value = 773 - Threshold Method = 2	0.331214	0.71571	0.958697	0.232151	0.553643	0.293562	0.099063	0.162231
3	132HighB.tif	Image Cal Value = 773 - Threshold Method = 2	0.348273	0.669618	0.927226	0.236944	0.504626	0.279525	0.111329	0.165167
4	132HighC.tif	Image Cal Value = 773 - Threshold Method = 2	0.338932	0.712736	0.956459	0.241704	0.549798	0.294496	0.097227	0.163111
5	135HighA.tif	Image Cal Value = 773 - Threshold Method = 2	0.41934	0.794515	1.008563	0.246819	0.566249	0.267016	0.172521	0.228495
6	135HighB.tif	Image Cal Value = 773 - Threshold Method = 2	0.389195	0.798011	1.010528	0.226226	0.56312	0	0.162969	0.235087
7	135HighC.tif	Image Cal Value = 773 - Threshold Method = 2	0.387799	0.80087	1.017338	0.22382	0.566052	0.296028	0.163979	0.235029
8	136HighC.tif	Image Cal Value = 773 - Threshold Method = 2	-1	1.068133	1.16869	0	0	0	0	0
9	136HighD.tif	Image Cal Value = 773 - Threshold Method = 2	0.492889	1.0379	1.153377	0.309496	0.78205	0.32831	0.183393	0.256061
10	137HighA.tif	Image Cal Value = 773 - Threshold Method = 2	0.40807	0.774164	0.996197	0.277659	0.589776	0	0.130411	0.184581
11	190HighA.tif	Image Cal Value = 773 - Threshold Method = 2	0.38337	0.750401	0.982618	0.22542	0.511295	0.304908	0.15795	0.239361
12	1connected2Border.tif	Image Cal Value = 773 - Threshold Method = 2	0.42575	0.831305	1.033179	0.290223	0.648183	0.292773	0.135527	0.183323
13										
14										

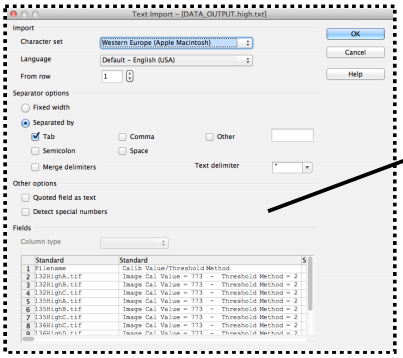
Opening Data Files in OpenOffice Spreadsheet

Open RootScan data file in OpenOffice spreadsheet by selecting 'Insert:Sheet From File...' in the OpenOffice main menu bar.

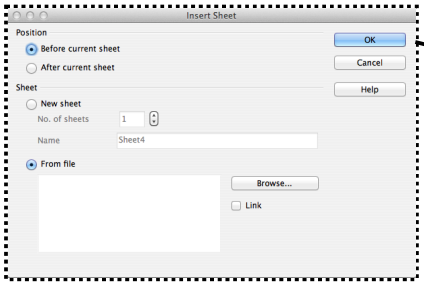


Selecting file from an example directory.



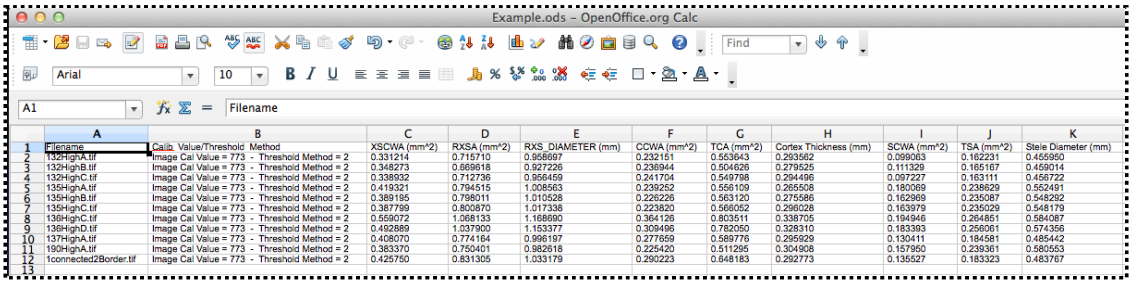


Text importation options allow the user to specify the character type and text separator options for the spreadsheet layout. Make sure the text separator options are set to the 'tab' separator.



Select the Ok when finished with selections

RootScan data shown in OpenOffice spreadsheet



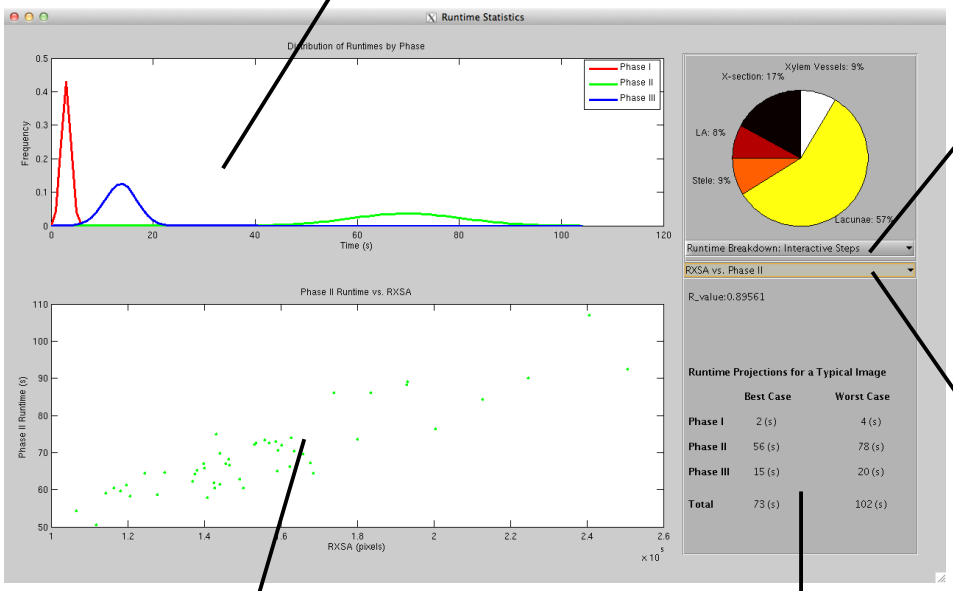
	A	B	C	D	E	F	G	H	I	J	K
1	Filename	Calib. Value/Threshold Method	XSCWA (mm^2)	RXSA (mm^2)	RXS_DIAMETER (mm)	CCWA (mm^2)	TCA (mm^2)	Cortex Thickness (mm)	SCWA (mm^2)	TSA (mm^2)	Stele Diameter (mm)
2	132HighA.tif	Image Cal Value = 773 - Threshold Method = 2	0.331214	0.715710	0.958697	0.232151	0.563643	0.293562	0.099063	0.162231	0.465950
3	132HighB.tif	Image Cal Value = 773 - Threshold Method = 2	0.348273	0.669618	0.927226	0.236944	0.504626	0.279525	0.111329	0.165167	0.459014
4	132HighC.tif	Image Cal Value = 773 - Threshold Method = 2	0.338932	0.712736	0.956459	0.241704	0.549798	0.294496	0.097227	0.163111	0.456722
5	135HighA.tif	Image Cal Value = 773 - Threshold Method = 2	0.419321	0.794515	1.008563	0.239252	0.556109	0.285508	0.180089	0.238629	0.552491
6	135HighB.tif	Image Cal Value = 773 - Threshold Method = 2	0.389195	0.798011	1.010526	0.226226	0.563120	0.275586	0.162969	0.235087	0.548292
7	135HighC.tif	Image Cal Value = 773 - Threshold Method = 2	0.387799	0.800870	1.017338	0.223820	0.566052	0.296028	0.163979	0.235029	0.548179
8	136HighC.tif	Image Cal Value = 773 - Threshold Method = 2	0.559072	1.068133	1.168690	0.364126	0.803511	0.338705	0.194946	0.264851	0.584087
9	136HighD.tif	Image Cal Value = 773 - Threshold Method = 2	0.492889	1.037900	1.153377	0.309496	0.782050	0.328310	0.183393	0.256061	0.574356
10	137HighA.tif	Image Cal Value = 773 - Threshold Method = 2	0.408070	0.774164	0.996197	0.277659	0.589776	0.295929	0.130411	0.184581	0.485442
11	190HighA.tif	Image Cal Value = 773 - Threshold Method = 2	0.383370	0.750401	0.982618	0.225420	0.511295	0.304908	0.157950	0.239361	0.580553
12	1connected2Border.tif	Image Cal Value = 773 - Threshold Method = 2	0.425750	0.831305	1.033179	0.290223	0.648183	0.292773	0.135527	0.183323	0.483767
13											

Additional Variables Analyzed During your Rootscan Session

RunTime Statistics

During your batch analysis session RootScan measures another important series of data points, namely runtime statistics. For each Phase, RootScan monitors the time required to analyze the steps contained within those phases for every image. These statistics can be used to project the time required for a typical directory. Once you have finished analyzing an image directory you can view the runtime statistics for that directory within the main RootScan window or view the data file externally in another program.

To view all runtime statistics within the main RootScan window, select the RunTime Statistics option under the Batch Analysis menubar item. You will be prompted to select the completed directory for which the runtime statistics are computed. The Runtime statistics module is shown below with all its features.



Normal distribution functions showing the general runtime template for the three phases in your directory.

Dropdown menu changes pie chart data.

Dropdown menu changes scatter plot data.

Scatter plot showing Time spent on Phase II (i.e. image binarization) for all images.

Runtime projections for typical images.