ROOT SCAN 2.0 USER MANUAL

1: LOADING AND MENUS

* Note the banner in the center of the screen first. Before loading any images, modify the grayscale channel by going to Options → Choose Grayscale Channel, choosing the channel you would like to use to transform the images to grayscale, and pressing ok. This must be done before loading images, because they will be transformed to grayscale upon loading.
* Note also the scale. This is the size in pixels per millimeter of the images. Modify the scale at any time before clicking "Finish" (in phase 3) by going to Options → Choose Scale, typing in the correct scale, and pressing ok.
* You may change the color that the objects and data will be drawn in by going to Options → Set Colors. Then click on the button with the name of the datum whose color you would like to change. Select your color, then press ok, and then ok again.
  + Only four colors are listed for cell files because after the fourth file the colors will repeat.
* The help menu has three options.
  + About Root Scan: This described the version number, the date completed, and the author.
  + Data Titles Key: This lists the abbreviations used in the data output and describes what each abbreviation means.
  + Keyboard Shortcuts: This lists the keyboard shortcuts that you can use and what button they will trigger.
* Loading an image
  + It is very important to load an image before starting the program. There are three ways to load images.
    - 1. File → Load Image. This will load a single image. If you are doing many images, it is more efficient to load an image set because some parts of the program are multithreaded.
    - 2. File → Load a Set of Images. This will load an entire folder of images. It is important that the folder only contain the images that you want to load, nothing more.
    - 3. File → Load from a Saved Session. This will load from a special folder that contains the details of a previously-saved session of Root Scan. It is important not to tamper with the folder that Root Scan wrote out when it saved. Simply load the folder, do not modify it. This will take you to the step that you were on when you saved last.
  + When an image or image set is loaded, all of the images are converted to grayscale and resized to fit the screen. This may take a while, especially if the images are large. It is not recommended to use images with dimensions larger than 500-700 pixels, as that will slow the program down without a great increase in accuracy. A very large set may take several minutes or longer to load. If it is taking too long, try a smaller chunk.
* You can save a session at any point by selecting File → Save. This will allow you to name the folder that you are saving. All images and data generated so far will be saved. Do not tamper with that folder. The program has populated it in a certain way and modifications may make your saved session unable to be loaded.
* You can quit by selecting File → Quit.

2: PHASE 1, SETUP

* Note that after loading you can see the first image of your set. Its name is in the lower left corner. In the lower right corner of the blue panel is a button that says Permanently Skip this Image. You may select that button at any time throughout the processing to remove that image from the set. In the output for the session all of the data of a skipped image will be -1 and it will have "<SKIPPED>" written in the comments.
* At the bottom of the yellow panel is a progress bar that shows what percentage of the total number of steps you have completed.
* Note that hovering over a button gives a brief description of what that button does.
* After loading an image or image set, press Start. Start will find the outline of the first cross section.
* Now you will cycle through all the cross sections, finding the outlines. Each outline is shown in yellow. You may click and drag any of the orange points on the line to modify the outline. You may also double click to add or remove a point from the outline. If the outline is too incorrect for you to salvage, you may click Discard and Draw Myself. This will allow you to click and drag to draw an ellipse. You may modify the ellipse as you can modify the program-generated outline (drag, double click, discard and draw again). When you are satisfied with the outline, click Approve Outline. You will go on to the next cross section, or go to the processing step.
* The program will crop the images to their outlines in order to decrease the size of the image and make the rest of the program faster.
* Once outlining is complete, select Process. This will threshold all of the images at once. It generates two thresholded images per cross section, one to analyze the cortex and one to analyze the stele. This is the longest step. For large sets, it may be possible to leave and return later while this step runs. The program will beep when processing finishes.
  + It is recommended not to save and try to reload until after processing, as saving reduces image quality for the original images, but not for the thresholded images that are exclusively used after processing.

3: PHASE 2, STELE

* Click Start Phase Two. This will find the stele outline of the first image in the set. You can modify the stele outline in the same way as you modified the cross section outline: drag, double click to add or remove, discard and redraw.
* Approve the outline by clicking Approve Outline. This will take you to the next cross section's stele outline or, if you've finished the set, to meta xylem vessel identification.
* You can do four actions to modify the xylem identifications:
  + 1. Click to change an object from a xylem vessel to a cell. Xylem vessels are blue and cells are pink. Initially only the blue xylem vessels are shown, but if you click in the stele you will be able to see the cells as well. Clicking within an object changes it back and forth.
  + 2. Merge objects by selecting Merge Objects. The button will switch to say Stop Merging. While merging, click within one of the objects you want to merge and drag to the other objects. You should see a small line connecting the newly merged objects. That means that they are now considered one. When you are done merging, click Stop Merging, or Approve Xylem Vessels. Both will stop you from merging.
  + 3. Draw an ellipse by selecting Draw Cells. Once selected, you will be able to draw on ellipse in the same way you drew an ellipse in Discard and Draw Myself for the outlines. The outline will be automatically considered a cell. You can click it to convert it to a xylem vessel if you would like.
  + 4. Right clicking inside an object will delete it entirely. This may be useful if you have concentric objects and the program doesn't realize you're trying to click the inner one - deleting the outer one will allow you to modify the inner one.
* Once you are satisfied with the xylem identification, click Approve Xylem Vessels. This will take you to the next cross section or to phase 3 if you've finished your set.

4: PHASE 3, CORTEX

* Begin by pressing Start Phase Three. This will find the aerenchyma for the first cross section. Finding the aerenchyma begins happening in the background as soon as you approve the stele outline. This should speed it up, especially for the latter images of the set
* Finding the aerenchyma will display the aerenchyma in red and the cells in pink. You can modify them in exactly the same ways as you modified the xylem: click to change, merge, draw cells, and delete. When you are satisfied with both the aerenchyma and the cortical cells, click Approve Aerenchyma.
* After approving the aerenchyma and cortical cells, you will go straight to the cell files. You will not do every image on aerenchyma and then do every image on files - each image goes through phase 3 to completion before you go on to the next image. This is to give the threads that are finding the aerenchyma in the latter images as much time as possible to finish before asking for their results.
* Each cell will have a color and its cell file written inside it. You can modify the files in two ways.
  + 1. Type into the text box labeled "File to edit" the number of the file that you would like to add cells to. Then click on cells in the image. Each cell you click on will be added to the file whose number is in the text box.
  + 2. Put your mouse on top of a cell, but don't click. Use the up and down arrow keys on the keyboard to increase or decrease, respectively, the file number of that cell. You cannot decrease below zero, but you can increase indefinitely.
* If you find the numbers distracting, you can select View without Numbers to remove the cell file numbers from the image. The button will change to View with Numbers, and you can select that to see the numbers again.
* Approve the files by selecting Approve the Files. Then you will be asked to click Finish. Finish will display the results in the correct units.

5: FINISH

* You will see a scrollable panel with the lists of data. The image will display some of the data you've collected. You can do several things to modify your data one last time.
  + All of the numbers are displayed in text boxes. If a number is wrong and you know what the right number is, you can click in the box and type in the correct number.
  + Beside each datum is a checked check box. If you uncheck the box, that datum will be deleted from the output of that cross section. You may find this useful if you know a number is wrong but you don't know what the correct number is.
  + You can click the button Edit Xylem at the bottom. This will allow you to click on objects in the stele and convert them from xylem to cells or vice versa. That is all you can do with Edit Xylem. When you are finished, be sure to click Done before moving on.
  + You can edit the lacunae in the same way as the xylem by selecting Edit Lacunae. Again, be sure to click Done when you are finished
* The Finish button will have changed to say Reset. Selecting Reset will reset all the data to the values that the program found.
* When you are satisfied with all the data, select Approve. This will take you to the beginning of phase 3 for the next cross section and you can repeat what you have done on that one. Or, if you have finished all the images, it will ask you to save the output. Name the file and click save. When you see the message "Your data has been saved", you can close the program.
* The output file that you save will be a CSV (comma separated vector). The first line will be the scale in pixels per millimeter that was used to generate the data. The second line will contain the abbreviations describing the data. Then you will have one line per image with all of its data, in the order that the second line describes it.