RSAJ Manual

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Summary

RSAJ is a project for the ObjectJ plugin for ImageJ. RSAJ was developed for the measurement of root system architecture traits in maize but the ideas can be extended to other species. Traits measured are intended to be the same or improvements as those measured in shovelomics. The objects can be modified to a user's specific situation. RSAJ allows the user to place markers on images, and to edit those markers later. The makers define objects such as points, lines, and angles. The user can review a project any time after completion to check the accuracy of measurements by seeing where the markers are located overlaid on images.

Image requirements

Images can be taken with any type of camera mounted above the root crown. Higher quality data will be generated from higher quality pictures in general. A matte, black background works well, such as a table painted with blackboard paint. A washed root crown should be placed on the background, with one nodal root cut off from that whorl and placed on the side of the image. Keep the stem of the root crown as vertical as possible. Including a scale of a known size is necessary to calculate pixels per length. Best practice includes keeping a tag with the sample identity in the picture as well, such as printed labels. Renaming image files to the sample ID is most convenient, as RSAJ will include the image name in the results. For maize, it is best to at least image the outside brace roots that penetrate the soil along with the most outside crown roots. Brace roots will be pigmented while crown roots will not be pigmented. Simply cut off a whorl of brace roots to reveal crown roots.

Opening RSAJ the first time and adding images to analyze

1. Install ImageJ using their directions and review online documentation

http://imagej.nih.gov/ij/

2. Install ObjectJ using their directions and review online documentation

http://simon.bio.uva.nl/objectj/

3. Download RSAJ ObjectJ package file to folder containing images

4. Open ImageJ, navigate to Plugins – jars – ObjectJ

5. Open RSAJ by ObjectJ – Project – Open Project, navigate to folder

6. Drag and drop images intended for analysis into the Images tab of the Project Window (note these images must be in the same directory as the RSAJ project file).

7. Before beginning, become familiar with the Project Window by reviewing the following pages.
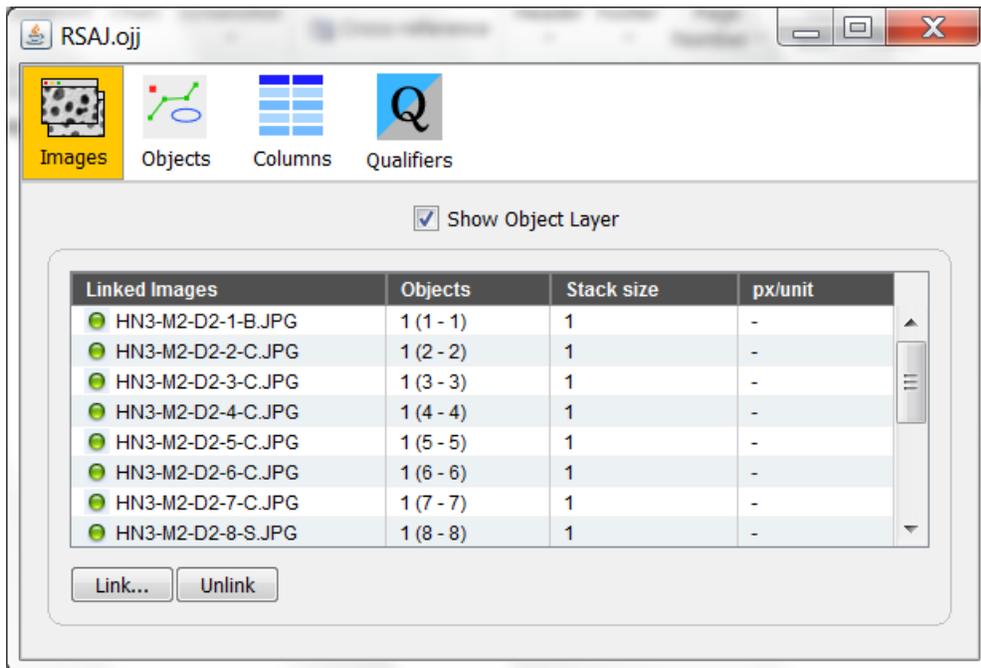
The Project Window consists of tabs for Images, Objects, Columns, and Qualifiers. We will not use the Qualifiers tab. The above screenshot shows the Images tab after images to be analyzed have been dragged and dropped inside. Alternatively, the Link button can be used. The images must be in the same folder as the project file.
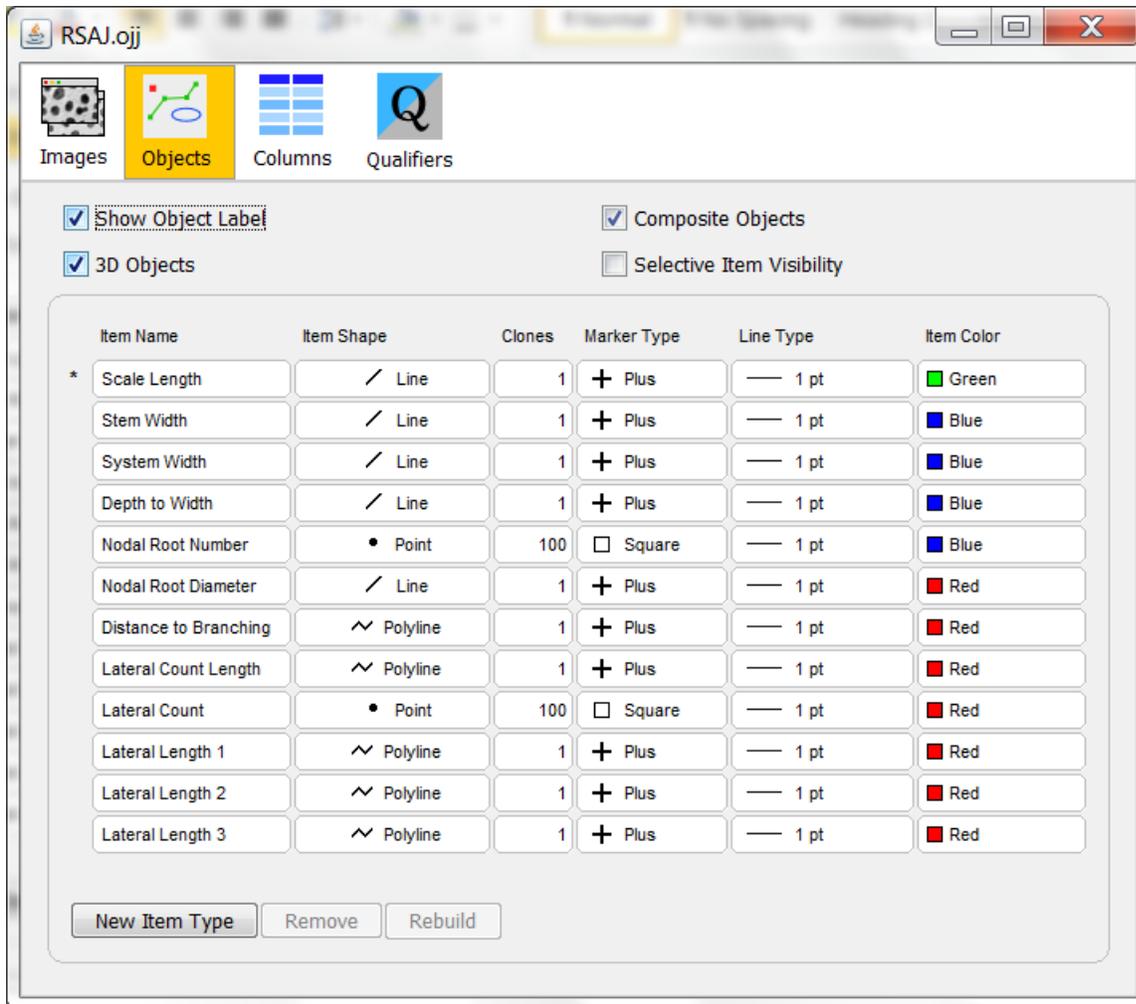
The Objects tab of the RSAJ project window shows all the traits that will be measured. The scale length is in green, root crown traits are in blue, and traits measured on an individual nodal root are in red. Clones show the numbers for each object, and for most we are only measuring one. Notice that the traits we count (nodal root number and lateral root count) have 100 clones. The clones for counting are just set high enough so you will never exhaust the possible number in one image, your data will only show the number you count. If you don't wish to measure a trait, just remove it from this list. However, nothing needs to be changed to use RSAJ as is. Traits are explained in the protocol, but the names are meant to be self explanatory.
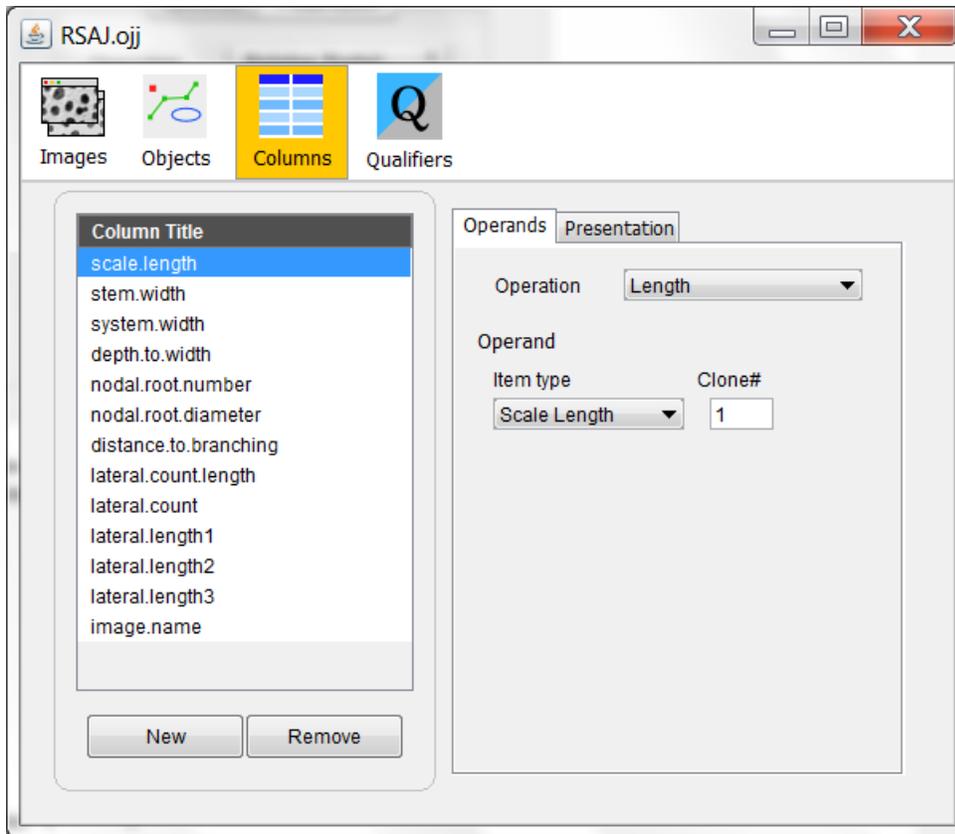
The Columns tab is where objects are interpreted as data via the Operation setting. These operations are used to output your objects to the results. The names of the columns in the data are similar to the names used on the Objects tabs and readily readable by R. Studying this will allow you to make your own length, count, and angle objects as needed. If you don't wish to measure a trait, just remove it from this list. However, nothing needs to be changed to use RSAJ as is.

Measuring root system architecture in maize using RSAJ

1. Double click on the first image to analyze

2. Measure the length of your scale. Click on one end, then the other to form a line. If the camera zoom and height is constant then you only need to do this for one image. However, it might be safe to always do it.

3. Automatically jumps to measure width of stem where the current whorl of nodal roots originates. Click on one edge of the stem, then the other to form a line. Try to measure exactly where the nodal roots farthest to the left and right intersects the stem on both sides and stay as perpendicular to the growth direction of the stem as possible. Review angle writeup.

4. Automatically jumps to measure root system width. Click on the left most nodal root then the right most nodal root to form a line. Measure the width as far towards the broken ends of the nodal roots as possible while keeping the line perpendicular to the growth direction of the stem and parallel to the stem width.

5. Automatically jumps to measure depth to width, or the height. Click in the center of the stem width line, then in the center of the root system width line, but make sure this line is parallel to the stem and perpendicular to the two widths.

6. Automatically jumps to count the nodal roots. Click on each visible nodal root in that whorl. Because the maize root system is symmetrical, the user can click on each nodal root again to estimate the total number of roots in that whorl. Zoom in before going to 7.

7. Press tab to advance to measures of the excised representative nodal root, starting with its diameter. Click on its left side then right side to form a line near its end that connected to the stem.

8. Automatically jumps to measure distance to branching. This is a polyline, which means each click yields a line segment, and length will be reported by summing the lengths of those segments. This allows us to better follow curves that may exist. First click on the end that was attached to the stem then add line segments by clicking while following the curve of the root. End the polyline where roots begin to emerge.

9. Press tab to advance to measuring the length over which you will count lateral roots. This is a polyline so you can follow the curve of the root over 2-4 cm.

10. Press tab to advance to lateral root counting. Click on each lateral root visible and originating from the area of root that you marked the length over which to count the laterals.

11. Press tab to measure lateral root length. Chose representative laterals and draw a polyline, and press tab to advance to the next lateral.

12. Close the image and start over from step 1 with the next image.

Tricks of the trade and hotkeys

Zoom in and out with the + and – keys. Press and hold spacebar to then click and drag the picture. With ObjectJ, it seems better to zoom in before advancing to the next measurement because it sometimes interprets zooming when on a new item that hasn't been measured as forming a new object. If this happens, then when you start measuring the next trait you will see another number form. If this happens, choose the finger tool from the ObjectJ tools and click on the last thing you measured to go back to editing that object then advance to the next trait. If you make a mistake here, all the data will be kept, and you will simply have to edit the dataset to move the data to the same row.

If you add an extra line segment to a polyline or an extra point when counting, use the backspace key to delete it before advancing to the next trait.

If you need to move a point after placing it, choose the move tool then hold down alt and click and drag the point you wish to move.

Sometimes you might find a window missing, you can allways bring them up from the ObjectJ menu in ImageJ, but might find these helpful:

Ctrl + shift + F1 = Project Window

Ctrl + shift + F2 = Tools

Ctrl + shift + F3 = Results, export to text from this window

R Script

A commented R script is included for converting pixel lengths to distances and for calculating angles using trigonometry. The user simply needs to rename their data to match the script and supply the known scale length in units they wish to have for lengths (cm is good).