MSU-RIPL Website Users Manual

(MSU-Root Image Processing Laboratory (RIPL)

A. Root Image Processing or washed roots (WR-RIPL) and minirhizotron roots (MR-RIPL)

1) **Staining procedures for enhancing RIPL sensitivity for your digital root files, below, and Appendix C, pp. 20ff**
2) **Root image processing of minirhizotron roots Appendix C, pp. 20ff**

B. Washing roots from mineral soils, Appendix A, pp. 7ff

C. Guidelines for Digital Scanning Washed Roots

D. Log on to MSU-RIPL Website for image processing digital root files.

F. Preprint publication by Kavdir and Smucker (2004), Appendix B, pp.10ff

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A. Root Image Processing or washed roots (WR-RIPL)

1. Sample Preparation and Staining procedures for enhancing RIPL sensitivity for your digital root files:

a. Put the sampled roots in a Whirl Pak® bag that contains 100ml of 20% methanol solution.

b. Dye the sampled root

- Dye solution: 2g of Malachite green in 100ml of 80% methanol
- Inject 0.5ml of dye solution using syringe and needle into a whirl-pak bag that has root sample and 100ml of 20% methanol solution.

c. Store the dyed roots for at least 2 days in 4 C for the most uniform distribution of the dye.

B. Washing roots from mineral soils

The best method for removing all roots, even the finest roots form <0.025 to 4mm across, from mineral soils is by the patented Michigan State University hydropneumatic elutriation system of separating roots and organic matter from mineral soils. This root washing system can be purchased from the Gillison's Fabrication Company, an independent manufacturer. Their website is: http://www.gillisons.com/

Also review operation manuals and best operation procedures outlined in Appendix A, below, p. 7-ff.

Reports of root removal method were first published in 1982 by Smucker, A.J.M., McBurney, S.L. and Srivastava, A.K., 1982. Quantitative separation of roots from compacted soil profiles by the hydropneumatic elutriation system. Agron. J. 74, 500-503.

Literally hundreds of journal reports can be reviewed at many journal websites. A computer search of book reports on the hydropneumatic elutriation system is available at the following website: http://print.google.com/books?q=hydropneumatic+elutriation&btnG=Search+all+books&lr=&ie=UTF-8

Roots washed by the Gillison Hydropneumatic Elutriation Root Washer are clean of mineral soils, but may also contain soil organic matter (SOM). This SOM can be removed if the roots plus SOM are stored in 20% butanol, menthanol, or other alcohols for at least 24 h. Then pour the entire sample into a tall gall beaker, add water and let the roots move to the bottom of the beaker. Decant off the undesired SOM and retain the roots. Be careful to remove any roots which may have grown through or are trapped by the SOM. If roots are desired to be stained, we suggest a Malachite Green or other cellular stain, as outlined above in A 1).

C. Guidelines for Digital Scanning Washed Roots

1. Scanning and saving digital files

a. Place 1-6 clear plastic trays, 15 x 15 cm, on the scanner.
b. Pour enough water, ~1mm, into each tray or until bottom of tray is covered.
c. Place the dyed root sample in the tray without overlapping.
d. Close the cover of scanner.
e. Scan and save the image as jpeg format at resolutions ranging from 300 to 400, unless you have very fine roots.

Table 1. Detectable root diameter and length according to scanning (dpi) resolutions.

Pix in^{-1} (Pix cm^{-1})	75 (29.53)	100 (39.37)	150 (59.06)	200 (78.74)	300 (118.11)	600 (236.22)	1200 (472.44)
Distance between 2 pixels (*u*m)	338.6	254.0	169.3	127.0	84.7	42.3	21.2
Distance between 3 pixels (*u*m)	677.3	508.0	338.6	254.0	169.3	84.7	42.3

2. Wire standards for MSU-RIPL:

Place 3-5 wires, bend them to look like roots, of the same diameters, that are 3-10 cm long, in the same transparent plate used for root imaging, and scan on a desk top scanner at 200 dpi. Our HP scanner used the following wires and calculations. Be sure to complete the same wire standards for your digital scanner and computer, to determine how many pixels per mm are applied to your scanning and computer combination as you develop root files to send to the MSU-RIPL. The MSU-RIPL website will ask you to enter the number of pixels per mm for your root files.

Scanner: HP model 6300C
File: Wire standards for MSU-RIPL
At 200dpi
9/10/04

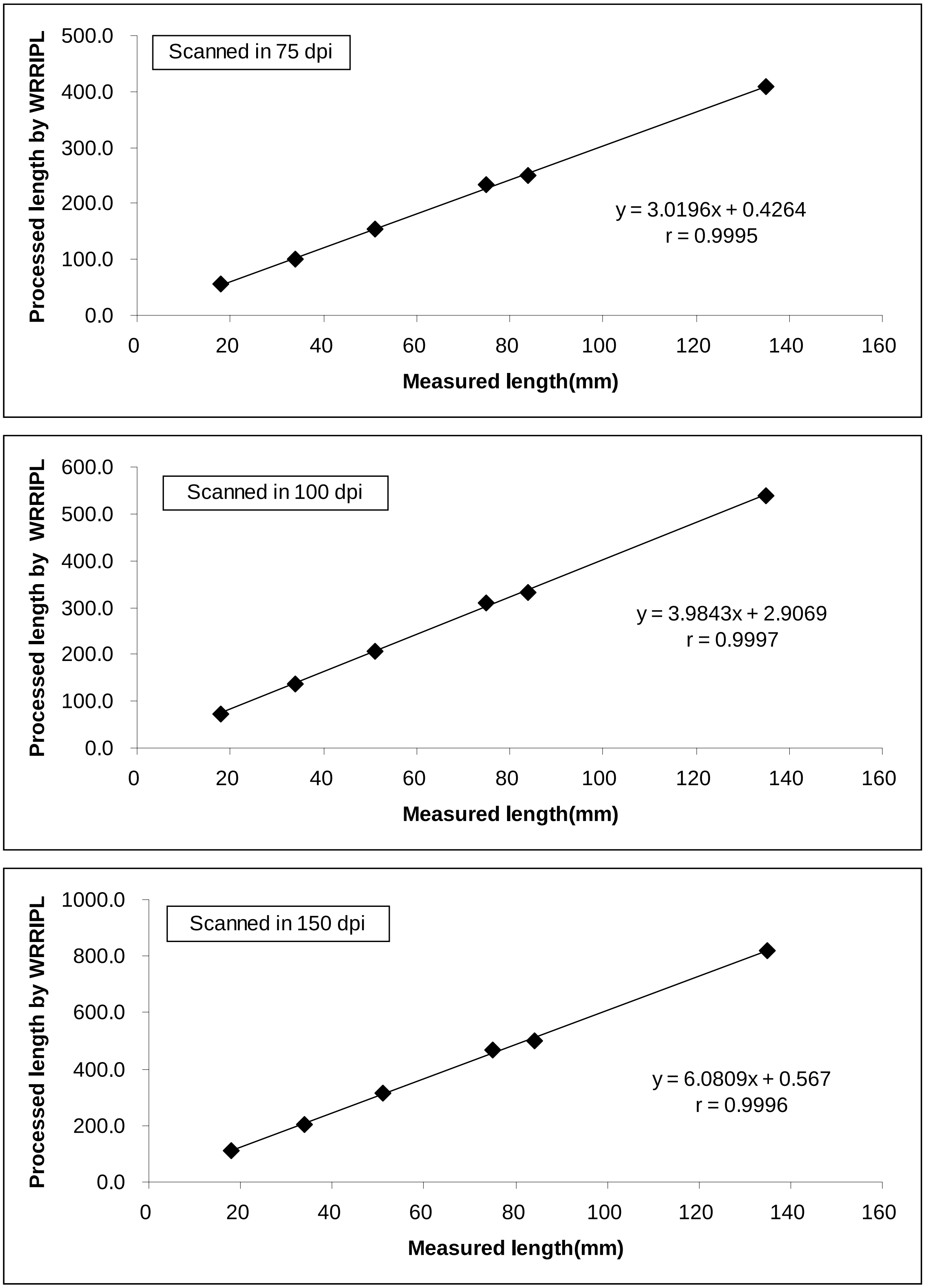
5 copper wires 0.5 mm diameter:
84, 72, 71, 71, 70 = 368 mm

Calculations:
Root length = **368 mm**
Root surface area = $2\pi rL$ = 6.28 x 0.25mm x 368 mm = **575 mm^2**
Root volume = $\pi r^2 L$ = 3.14 x 0.0625mm^2 x 368mm = **72.22mm^3**

3. Some background information for scanning wire standards

Table 2. Comparison of manually measured wire lengths and scanned, processed length by WR-RIPL at 3 different dpi resolutions.

No.	Meaured length (mm)	**Processed length by "WRRIPL"(mm) : whthout debris**						
		75pix/in 29.53pix/cm	100pix/in 39.37pix/cm	150pix/in 59.06pix/cm	200pix/in 78.74pix/cm	300pix/in 118.11pix/cm	600pix/in 236.22pix/cm	1200pix/in 472.44pix/cm
1	18.0	56.22	72.78	110.06				
2	34.0	99.55	137.11	201.75				
3	51.0	155.11	207.57	315.87				
4	75.0	233.09	309.32	466.39				
5	84.0	249.38	332.99	502.31				
6	135.0	408.00	539.45	821.15				



<Fig. 1. Evaluation of wire lengths of digitally scanned wires, 0.5 mm diameter, and analyzed by the WRRIPL command at 3 dpi resolutions. Correlations are comparisons between manually measured and image processed by MSU-RIPL computer.

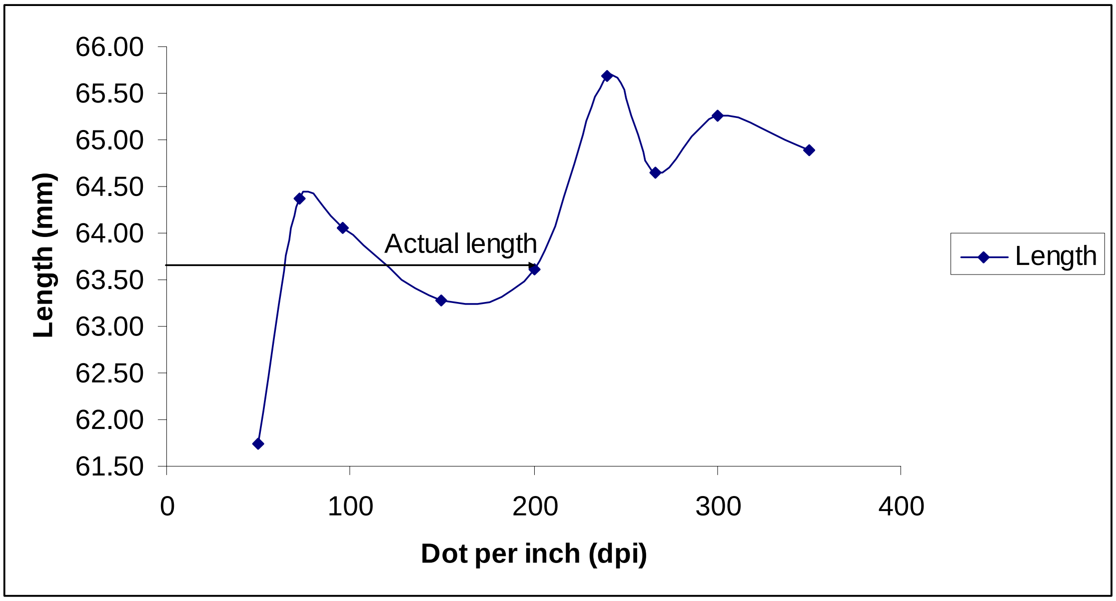


Figure 2. Estimated lengths of a 63.6 mm wire using MSU-RIPL software. Scanned images were made at 9 different dpi resolutions, black and white, and saved as JPEG files before submitting to the MSU-RIPL website. Note that 200 dpi appeared to give the best estimate of actual length. This will change depending upon your scanner.

The full manuscript, published by Kavdir and Smucker (2004) is listed below, p. 10ff.

D. Logging on to the MSU-RIPL Website for image processing digital root files

Point your browser to http://rootimage.msu.edu/ Click on the "Root Image Analyzer" If you are a new user, click on the "New User" button. If you have already registered enter your username/password and click the "Submit" button.

The "New User" button pulls up a form asking for a first and last name as well as an email address. A temporary password will be assigned and mailed to your email address. After the assigned password arrives, return to the main screen and log in, using the same user name and newly assigned password.

After logging in, you can upload files for analysis. JPEG or SUN RASTER files can be submitted singly, or several images can be combined in a ZIP or TAR.GZ file. The "More" button extends the form with more spaces for additional file uploads. The "Submit" button starts the upload. The "Change Password" button allows you to change your temporary password to a new password that you may wish to use.

After the "Submit" button is clicked the files are uploaded, and analyzed. When the analysis is finished, an email message is sent to your email address which you specified. The email notice will contain a link to a web site where the results can be viewed, and downloaded. The results will remain on the website for approximately 20 days.

E. References relating to the use and data developed by the MSU RIPL:

1. Liisa M. Pietola and Alvin J.M. Smucker. 2005. Elimination of non-root residue by computer image analysis of very fine roots. Soil Sci. Soc. of Finland J. (Accepted)

2. Kavdir, Y and A.J.M. Smucker. 2004. Comparison of root image processing programs for quantifying plant root parameters. *In:* Natural Resource Management for Sustainable Development. Proceedings of the International Soil Congress, June 2004, Erzurum, Turkey (See Appendix B, below.

3. Smucker, A.J.M. 2003. Root carbon contributions to soil aggregate formation and function. *In*: Roots: Dynamic Interface between Plants and the Earth. Kluwer Academic Pub, Netherlands. pp 421-426.

4. Pietola, L. and A.J.M. Smucker. 1998. Fibrous carrot root responses to irrigation and compaction of sandy and organic soils. Plant and Soil 200: 95-105.

5. Dowdy, R. H., A.J.M. Smucker, M. S. Dolan, and J. C. Ferguson. 1998. Image processing separation of soil debris from plant roots washed from soil cores by elutriation. Plant and Soil 200: 91-94.

6. Smucker, A.J.M. 1990. Quantification of root dynamics in agroecological systems. Remote Sens. Rev. 5, 237-248.

7. Smucker, A.J.M. 1993. Soil environmental modifications of root dynamics and measurement. Annu. Rev. Phytopathol. 31, 191-216.

8. Smucker, A.J.M., Ferguson, J.C., DeBruyn, W.P., Belford, R.L. and Ritchie, J.T., 1987 Image analysis of video recorded plant root systems. Spec. Publ. Am. Soc.. Agron. 50, 67-80.

9. Smucker, A.J.M., McBurney, S.L. and Srivastava, A.K., 1982. Quantitative separation of roots from compacted soil profiles by the hydropneumatic elutriation system. Agron. J. 74, 500-503.

If new problems arise, contact: smucker@msu.edu for additional information, when needed.

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Appendix A

Root Imaging, Washing and Computer image processing at Michigan State University

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The MSU Root Image Processing Laboratory (MSU-RIPL) is readily available at the present time on the following website: http://rootimage.msu.edu/root/index.php

Logon and receive a password and send wire standard and root sample files. The RIPL will identify length, surface areas, and volumes of roots having multiple diameters. These multiple diameters are also indicators of root branching, yet must be modified based upon the genotype or tree species being measured.

If you use a flatbed scanner to digitize your washed root images, we suggest the following protocols:

Four requirements for the best root imaging and processing are:

1. Place all root samples at least 5 mm from the containers edge, so that the root samples can be highlighted and selected by the operator in your lab, before sending each image to the MSU-RIPL.

2. Neither root samples nor wire standards should not overlap and they should be placed in water films thick enough to cover the entire root sample without excessive floating. This minimizes the false edge effects of a water meniscus along each root sample.

3. Save digital images in either JPEG or TIFF formats.

4. Be sure to use the pixels per mm established by the MSU-RIPL results from the wire standards sent and following the receipt of standard wire data, before submitting unknown root samples.
Attached are some additional suggestions for the most quantitative evaluations of washed roots.

The following publications have brought this RIPL, along with minirhizotron and root washing into the 21st century. These may be more than you asked for. Therefore, I placed and (*) on the most informative.

References: Note most illustrative references are identified by a (*)

<u>Commercially available hydropneumatic elutriation root washer that recovers up to 99.8% of fine roots:</u>

*Smucker, A.J.M., S.L. McBurney and A.K. Srivastava. 1982. Quantitative separation of roots from compacted soil profiles by the hydropneumatic elutriation system. Agron. J. 74:500-503.

Srivastava, A.K., A.J.M. Smucker and S.L. McBurney. 1982. An improved mechanical soil-root sampler. Transactions of the ASAE, Vol. 25, No. 4, pp. 868-871.

Sales website: http://www.gillisons.com/root_washer.htm

Minirhizron camera recording:

*Box, J.E., Jr., A.J.M. Smucker and J.T. Ritchie. 1989. Minirhizotron installation techniques for investigating root responses to drought and oxygen stresses. Soil Sci. Soc. Amer. J. 53(1):115-118.

Ferguson, J.C. and A.J.M. Smucker. 1989. Modifications of the minirhizotron video camera system for measuring spatial and temporal root dynamics. Soil Sci. Soc. Am. J. 53(5):1601-1605.

Smucker, A.J.M. 1990. Quantification of root dynamics in agroecological systems. *In*: Instrumentation for Studying Vegetation Canopies for Remote Sensing in Optical and Thermal Infrared Regions, Remote Sensing Reviews. V.S. Goel and J.M. Norman, eds. 5(1):237-248.

McLean, M., G.S. Howell and A.J.M. Smucker. 1992. A minirhizotron system for *In Situ* root observation studies of Seyval grapevines. American J. Enol. Viticulture 43(1):87-89.

Smucker, A.J.M. 1993. Soil environmental modifications of root dynamics and measurement. Annual Rev. Phytopathol. 31:191-216.

*Murphy, J.A., M.G. Hendricks, P.E. Rieke, A.J.M. Smucker and B.E. Branham. 1994. Turfgrass root systems evaluated using the minirhizotron and video recording methods. Agron. J. 86(2):247-250.

Murphy, S.L. and A.J.M. Smucker. 1995. Evaluation of video image analysis and line intercept methods for measuring root systems of alfalfa and ryegrass. Agron. J. 87(5):865-868.

*Pietola, L.M. and A.J.M. Smucker. 1995. Fine root dynamics of alfalfa after multiple cuttings and during a late invasion by weeds. Agron. J. 87(6):1161-1169.

Smucker, A.J.M., B.G. Ellis and B.T. Kang. 1995. Alley cropping on an alfisol in the forest savanna transition zone: Root, nutrient, and water dynamics. Agroforestry, pp. 103-121.

Minirhizotron sales website: http://www.bartztechnology.com/ucgi-bin/bartz/index.html

Computer image processing of washed (WR-RIPL) and minirhizotron (MR-RIPL) files:

Website: http://rootimage.msu.edu

References:

*Smucker, A.J.M., J.C. Ferguson, W.P. DeBruyn, R.K. Belford and J.T. Ritchie. 1987. Image analysis of video-recorded plant root systems. *In*: Minirhizotron Observation Tubes: Methods and Applications for Measuring Rhizosphere Dynamics. S.A. Barber and C.D. Boulden, eds. Agron. Soc. of Amer. Special Publication No. 50, pp. 67-80.

Smucker, A.J.M. 1988. Video recording and image analyses of the rhizosphere. Yearbook of Science and Technology. McGraw-Hill. 3 pp.

*Huang, C., A.K. Jain, G.C. Stockman and A.J.M. Smucker. 1992. Automatic image analysis of plant root structures. Proceedings of the 11th IAPR Conference on Pattern Recognition Methodology and Systems. The Hague, The Netherlands. pp. 1-8.

Huang, Q., A.K. Jain, G.C. Stockman and A.J.M. Smucker. 1992. A new perspective on segmentation: Token-based grouping at multiple levels. Image Processing: Theory and Applications on Segmentation, pp. 1-9.

*Majdi, H., A.J.M. Smucker and H. Persson. 1992. A comparison between minirhizotron and monolith sampling methods for measuring root growth of maize (*Zea mays* L.). Plant and Soil 147:127-134.

*Dowdy, R.H., A.J.M. Smucker, M.S. Dolan and J.C. Ferguson. 1998. Automated image analyses for separating plant roots from soil debris elutriated from soil cores. Plant and Soil 200:91-94.

Fahey, T.J, C.S. Bledsoe, F.P. Day, R.W. Reuss and A.J.M. Smucker. 1999. Fine root production and demography. *In*: Standard Soil Methods for Long Term Ecological Research. G.P. Robertson et al., eds. Oxford University Press, New York, NY. pp. 437-455.

Appendix B

F. Publication by Kavdir and Smucker (2004):

COMPARISON OF ROOT IMAGE PROCESSING PROGRAMS FOR QUANTIFYING PLANT ROOT PARAMETERS

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The aim of this study was to evaluate and compare two different web-based root image processing programs. Artificial roots from wires and drawn lines with known actual lengths and diameters were scanned and digitized using a flatbed scanner. Two web-based image processing programs, the MSU-Root Image Processing Laboratory (RIPL) and Scion 4.02 for Windows, were used to determine artificial lengths and areas of artificial standards and wheat root lengths and areas. Comparisons required flatbed scanning at similar dpi for both web-based image processors. Results showed a very close relationship between dpi of scanned materials, computer software and size of objects measured. These studies revealed that more accurate length and diameter estimates of roots were achieved by the MSU-Root Image Processing Laboratory (RIPL) than the Scion 4.02 for Windows. In addition to length and diameter, the RIPL web-based root image processing facility offers total volume dimensions for root diameters ranging from 0.127 to at least 10 mm. as well as the removal of non-root debris from images.

INTRODUCTION

Most models describing the transport of water and ions from roots to shoots require that water and ion flow be represented as rates per unit root surface area (Campbell, 1991). Root length is also used to characterize root development in relation to shoot growth, nutrient uptake etc. Directly measuring root length is time consuming and labor intensive. However image analyzing systems provide quick measurement for these root parameters. For example using public software together with a flatbed scanner, root length measurements can be completed in couple minutes. Prior studies measuring root parameters have used line intercept (Newman, 1966; Harris and Campbell, 1989), chain method (Pan and Bolton, 1991) etc. and using each of these methods were labor and time consuming. The latter methods used edge discrimination method (Pan and Bolton, 1991), the edge chord algorithm (Ewing and Kaspar, 1995) and scanning procedure (Bouma et al., 2000; Murphy and Smucker, 1995) but some of these methods were expensive. However, current public domain software (Scion Image) and the web-based root image processing system MSU-RIPL use common flat bed desk-top scanners for digitizing root and other fine filaments. The aim of this paper is to evaluate the accuracies of both systems and to compare their outputs with measurements of visual standards.

MATERIALS and METHODS

Creating a Digital Image of Root . Individual roots, wires and hairs with known length and estimated surface areas were scanned into digital format using EPSON Perfection 1260 desktop scanner images scanned at 150 dpi for SCION software and 200 dpi for MSU-RIPL software. The scanner and the software were operating on Desktop computer with Pentium III processor with128 MB RAM. An object was placed on the scanner; the lid of the scanner was closed, and a preliminary scan was made using the preview feature of the software. The preliminary image was converted from color to grayscale. The highlight and shadow levels within the exposure adjustment (selected from the tools menu) were manipulated to create a black image on a white background. Care was taken to not exclude actual edges of the materials scanned. The final version was saved as a TIFF file because GIF and JPEG compression are not compatible with SCION image analysis software. The same objects were scanned again at 200 dpi and file was saved as a JPEG format for MSU-WR-RIPL software.

Measurements using SCION 4.02:

We used public domain software (Image 4.02 for Windows, National Institutes of Health, Bethesda, MD) to measure the surface area of objects in a digital format. This program is available for Apple and Windows operating systems, and as of 14 May, 2002, could be downloaded at the following addresses: Apple

(http://rsb.info.nih.gov/nih-image/) and Windows (http://www.scioncorp.com). We opened the TIFF file to be analyzed within the Image software, and using the set scale option (selected from the analyze menu), we selected a unit (mm) to convert pixels to a unit of measurement. We included a standard of known dimensions (a ruler) within the image for calibrating the pixel conversion. Once the units had been selected, the grayscale-image was adjusted so that the image was composed of only black and white. Within the map box (selected from the Windows menu), a threshold option was selected to convert all color values to either a one (black) or zero (white). After that all roots and wires became white and background became black. Scion image software can only detect the area of black color. Within the File menu, the invert option was selected to convert white images to black images and vice versa. Then surface area of objects were calculated by selecting the measure option (selected from the analyze menu).

Measurements using MSU-RIPL software :

Using this software requires registration, after registration we have received login and password. Web page of WR-RIPL software is: http://rootimage.msu.edu/ and click on Root Image Analyzer. After logging on the page, we have clicked on washroot icon WR-RIPL. Values of pixels per mm for scanned images at 200 dpi was set to 7.874. We browsed image file from the browser icon and then clicked on submit button. Results have been sent to our e-mail addresses about couple minutes later. One of the outputs sent by MSU-RIPL program is presented in Table 1. Results are in pixels and to convert those to metric units we need to convert pixels to mm or cm. For instance if image is scanned at 200 dpi the conversion rate is equal to 7.874 pixels mm^{-1}. Thus length values need to be divided to 7.874 to convert lengths to mm.

Table 1. Root parameters results output of MSU-RIPL software.

<table>
<tr>
<td>Filename.JPG</td>
<td>cumulative statistics for 3 root segments
| total length | total surface area | total volume |
| with debris no debris | with debris no debris | with debris no debris |
| 444.87 413.69 | 10601.26 10418.87 | 263529.14 262403.24 |
total length per class | with debris no debris |
| class 0 [0-- 5] | 0.00 0.00
| class 1 [5-- 10] | 31.18 0.00
| class 2 [10-- 16] | 0.00 0.00
| class 3 [16-- 23] | 0.00 0.00
| class 4 [23-- 31] | 413.69 413.69
|
creating resulting output image
JPEG Header read: width 405; height 318; out color components 3; # of colors 0
peak 242, setting boundaries...
JPEG Header info: width 405; height 318; in_color_space RBG; input components 3
done</td>
</tr>
</table>

We compared the accuracy and precision of root area and length estimates from the desk-top scanner with estimates by hand measurements. Separate tests were conducted using metal wire and hair of known lengths, and multiple wire and hairs with known lengths.

Results and Discussions

Our previous (Kavdir, 2000) and current research (Figure 1) show that the accurate root length can be obtained when images were scanned at 7.874 p mm^{-1} (200 dpi). Bauhus and Messier (1999 and 2003) found overestimation of length when the resolution pixel size is smaller than 15% of the diameter of imaged object and similarly reported that appropriate scanner resolution for roots 1 mm diameter and larger is 11.8 pixels mm^{-1} or less.

Length measurements of objects by MSU- RIPL were very close to those measured manually. With the current SCION 4.02 program, only free hand tool can be used for length measurement but it is very time consuming for the real root measurements. Recently macro program was developed for Scion 4.02 by Kimura et al. (2001) and Kimura and Yamasaki (2003) for root length measurements. Unfortunately, in this research evaluation of this macro-program was not possible. Surfaces areas of objects such as leaf were very accurately measured by SCION 4.02. However, in the case of roots which have cylindrical shape, this program was limited to measure only one side of the roots. On the other hand MSU-RIPL assumes that roots have cylindrical shape and it calculates its real surface area. SCION did not give the correct results of thick plant roots, thick wires or ropes. (Table 2). When objects became thicker SCION software could not correctly predict the surface areas of objects. Results from Table 2 show that actual hand measured surface area of rod with 52 mm in length and 6.7 mm in radius was 1165 mm^2. If we assume that this object is rectangular therefore the surface area of it will be 348.4 mm^2. SCION 4.02 predicted very close to this value that was 346.95 mm^2. In reality, actual roots are not rectangular and surface areas must be calculated as cylinders. Result of surface areas measured by MSU-RIPL were 1318 mm^2. It is nearly 4 times greater than the one calculated by SCION. Length measurement is predicted as 52.52 by MSU-RIPL and 52.50 by SCION 4.02.

Table 2. Comparisons of thick wire length and area measured by hand, MSU-RIPL and SCION 4.02.

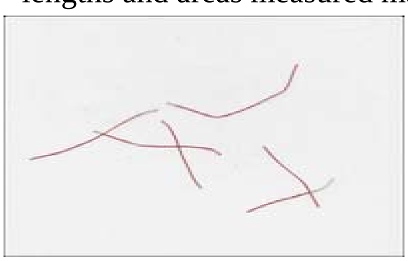
Original Image

Model Image

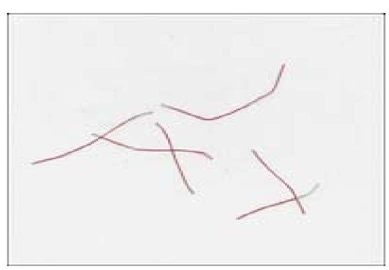
Measured by hand		Measured by WR-RIPL 200dpi		Measured by SCION 4.02 150 dpi	
Length (mm)	Area (mm^2)	Length (mm)	Area (mm^2)	Length (mm)	Area (mm^2)
52 52	1165 (cylinder) 348.4 (rectangular)	52.53	1318	52.5	346.95
Difference		1.0%	13.1%	0.9%	70.2%

When digital objects were very fine roots, area results obtained by the Scion 4.02 software approached to root areas measured manually and by MSU-WR-RIPL program (Tables 3 and 4).

Table 3. Comparisons of values produced by MSU-RIPL and SCION 4.02 with multiple thin wires lengths and areas measured manually.



Original Image



Model Image

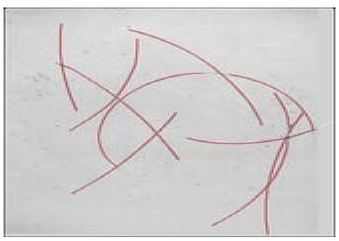
Measured by hand	Measured by WR-RIPL	Measured by SCION 4.02

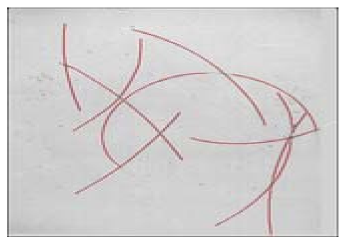
		200dpi		150 dpi	
Length (mm)	Area (mm^2)	Length (mm)	Area (mm^2)	Length (mm)	Area (mm^2)
139	33.36	139	32.41	NA	32.37
ERROR		0.0%	2.9%	NA	3.0%

NA: Not applicable (needs free hand measurement)

For fewer amounts of roots SCION 4.02 can be used but for very tiny and large quantities of roots it would be very time consuming. Because of manual measurements are required as designated by the NA was written in length column in Tables 3 and 4.

Table 4. Comparisons of hair lengths and areas measured by hand, MSU-RIPL and SCION 4.02.


Original Image


Model Image

Measured by hand		**Measured by WR-RIPL 200dpi**		**Measured by SCION 4.02 150 dpi**	
Length (mm)	Area (mm^2)	Length (mm)	Area (mm^2)	Length (mm)	Area (mm^2)
335	70.35	300	77.3	NA	46.52
Difference		10.4%	9.9%	NA	33.9%

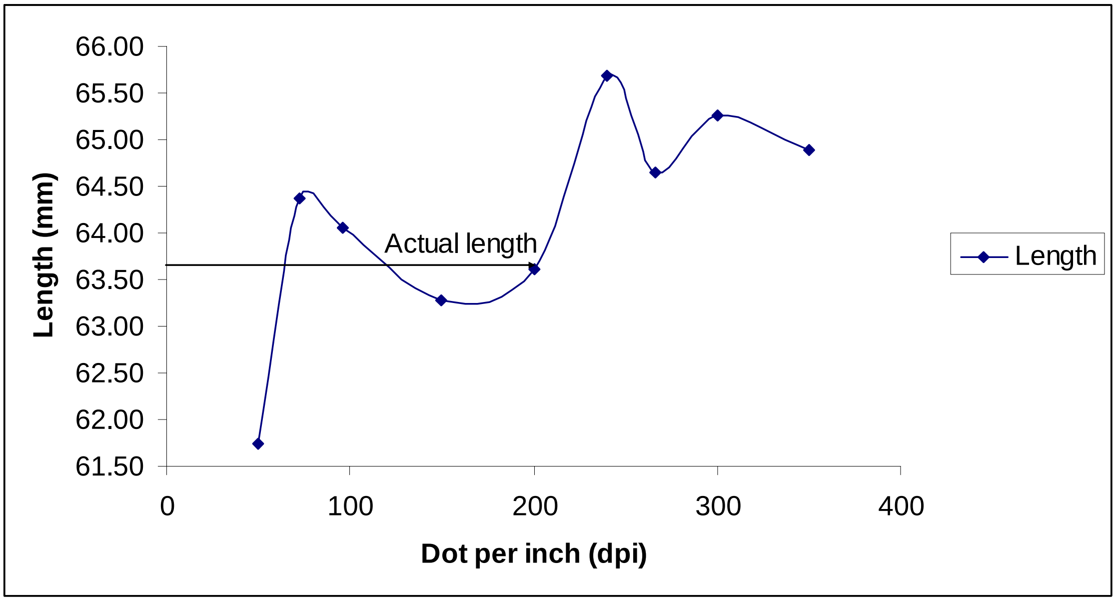


Figure 1. Estimated lengths of a 63.6 mm wire using MSU-RIPL software. Scanned images were made at 9 different dpi resolutions, black and white, and saved as JPEG files before submitting to the website.

Table 5. Table 2. Comparisons of wheat root parameters measured by MSU-RIPL and SCION 4.02.

Measured by WR-RIPL 200dpi		**Measured by SCION 4.02 150 dpi**	
Length (mm)	Area (mm^2)	Length (mm)	Area (mm^2)
475.42	1184.01	NA	291.66

Table 5 shows the images and results for the actual wheat root lengths and areas.

CONCLUSIONS

Two web-based image processing systems were compared using both manally measured materials and actual plant roots of unknown total root lengths and diameters. Although the SCION 4.02 appears to be useful to both Windows and Apple operating systems, the imaging software is limited to surface area measurements which are often erroneous for plant roots. In contrast, the MSU-RIPL imaging software offers more accurate root length and surface area values. Although not compared in this study, the MSU-RIPL web-based root imaging system offers total volume analyses of roots and cylindrical fibers as well as debris elimination options for plant root samples with up to 20% non-root like residues are present (Dowdy, et al., 1998).

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Appendix C

Root Imaging, Washing and Computer image processing at Michigan State University

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The MSU Root Image Processing Laboratory (MSU-RIPL) is readily available at the present time on the following website: http://rootimage.msu.edu/root/index.php

Logon and receive a password and send wire standard and root sample files. The RIPL will identify length, surface areas, and volumes of roots having multiple diameters. These multiple diameters are also indicators of root branching, yet must be modified based upon the genotype or tree species being measured.

If you use a flatbed scanner to digitize your washed root images, we suggest the following protocols:

Four requirements for the best root imaging and processing are:

1. Place all root samples at least 5 mm from the containers edge, so that the root samples can be highlighted and selected by the operator in your lab, before sending each image to the MSU-RIPL.

2. Neither root samples nor wire standards should not overlap and they should be placed in water films thick enough to cover the entire root sample without excessive floating. This minimizes the false edge effects of a water meniscus along each root sample.

3. Save digital images in either JPEG or TIFF formats.

4. Be sure to use the pixels per mm established by the MSU-RIPL results from the wire standards sent and following the receipt of standard wire data, before submitting unknown root samples.
Attached are some additional suggestions for the most quantitative evaluations of washed roots.

The following publications have brought this RIPL, along with minirhizotron and root washing into the 21st century. These may be more than you asked for. Therefore, I placed and (*) on the most informative.

References: Note most illustrative references are identified by a (*)

Commercially available hydropneumatic elutriation root washer that recovers up to 99.8% of fine roots:

*Smucker, A.J.M., S.L. McBurney and A.K. Srivastava. 1982. Quantitative separation of roots from compacted soil profiles by the hydropneumatic elutriation system. Agron. J. 74:500-503.

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Sales website: http://www.gillisons.com/root_washer.htm

Minirhizron camera recording:

*Box, J.E., Jr., A.J.M. Smucker and J.T. Ritchie. 1989. Minirhizotron installation techniques for investigating root responses to drought and oxygen stresses. Soil Sci. Soc. Amer. J. 53(1):115-118.

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Minirhizotron sales website: http://www.bartztechnology.com/ucgi-bin/bartz/index.html

Computer image processing of washed (WR-RIPL) and minirhizotron (MR-RIPL) files:

Website: http://rootimage.msu.edu

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