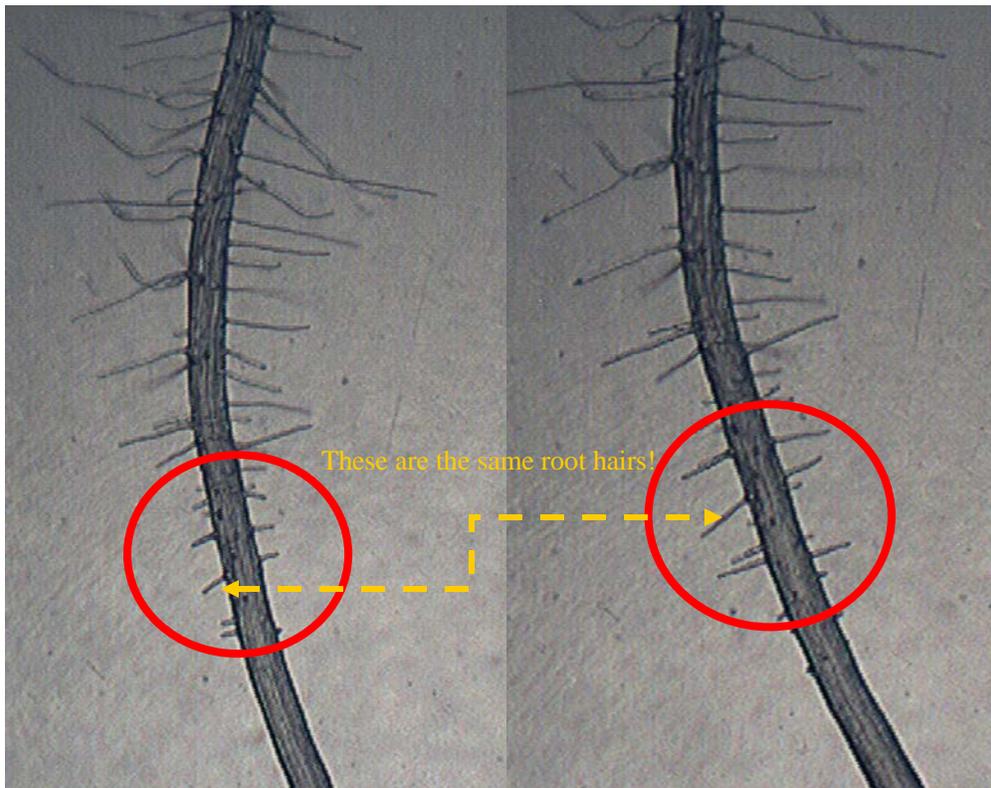


Image t0

Image t1



Above are images of the same root, taken 60 min apart (t0 and t1).

The red circles roughly represent the zone in which root hairs are actively growing. Using ImageJ as a tool for measurement, measure a root hair in the red circle on the t0 image. Then, find that same root hair on the t1 image and measure it. To determine growth rate, take the difference in length and divide by the time between the two images. (in this case, 60min) Repeat this for every root.

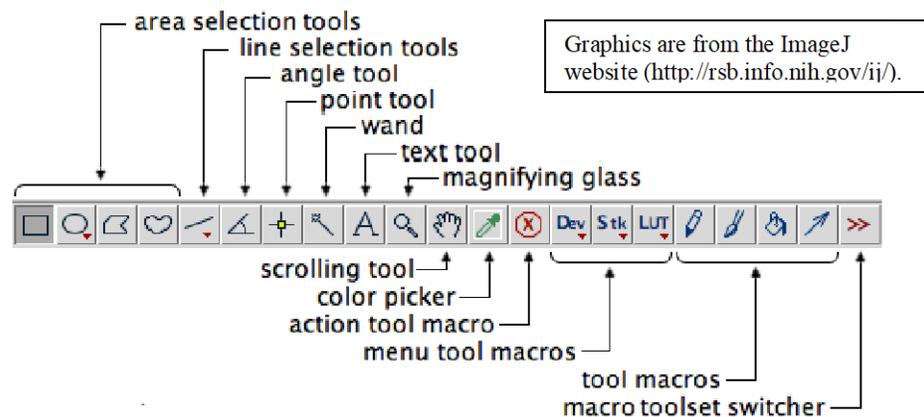
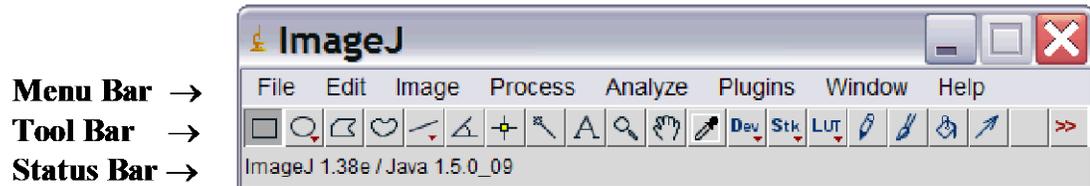
Remember, only select root hairs that are in the zone in which root hairs grow! Do not include root hairs greater than 100 μm in the t0 image. Because they are so long, they tend not to grow anymore!

How to use Image J

ImageJ is a powerful image analysis program that was created at the National Institutes of Health (NIH). It is in the public domain, runs on a variety of operating systems and is updated frequently.

Downloading ImageJ and setting scale:

1. Download Image J from <http://rsbweb.nih.gov/ij/>
2. Open Image J



3. File > Open and select your first image (time 0) that you are going to measure.
4. Analyze > Set Scale.
 - o Distance: enter number posted on the computer/microscope system you used
 - o Known Distance: 100
 - o Pixel Aspect Ratio: 1.0
 - o Unit of Length: "µm"
 - o Check "Global"
 - o Click Ok.
5. Look on the tool bar and make sure that the straight line "straight line selections" is selected. Right click on the icon and select straight line selection. However, segmented lines can also be used.
6. If you need to increase the size of the picture to get a better view, select the magnifying glass. You can increase the size by clicking on the photo. To zoom out, right click on the picture.
7. You are now ready to begin measuring.

Shortcuts:

- Measuring: After measuring a root, hit "M" on your keyboard. The length is displayed in a new data window.
- Make sure your root hairs at TIME 0 are greater than 0.2 µm and less than 100 µm
- Enlarging the Picture: Press Ctrl + and Ctrl - to shrink the picture.

- If you are comfortable with image J, it is possible to open two Image J programs at once so you can consecutively measure the roots for time 0 and time 60 of each plant. Remember to set the scale for BOTH Image J programs

Saving Results:

- Select the “Edit” tab, select “Select All,” select “Copy All,” and paste results directly into MS Excel worksheet.
- Save the Excel file, label with the date of the experiment and your names

Analysis

- Label your columns in Excel
- Determine the average length (use the command “=average ()”), Standard Deviation (use the command “= stdev ()”) and p-value in the t-test
 **for the t-test use command “=ttest(see below)”
 Array 1 = Column “A”
 Array 2 = Column “B”
 Tails = 2
 Type = 3 (two sample, unequal variance)

Tips:

- To keep up with hairs you have already measured, you can use the paint brush icon to place black dots next to the hairs you have already measured. It is located to the left of the paint can on the tool bar.

Troubleshooting:

- If you are measuring your root hairs and find that when you select “measure” the “length” value is not showing, it may be because the wrong icon is selected. Make sure that the “straight line selections” (the straight line) icon is selected.

Helpful Hints and Guidelines: Data Sheet

Excel Data Sheet:

What values should you have in your Excel data sheet?

- n-value (# of roots obtained per plate)
 - What is a good value? ~30 root hairs TOTAL FOR EACH PLATE. This means about 30 root hairs from the control plate and about 30 root hairs on each treatment plate.
- Growth rate: $(T1-T0)/60^* = \mu\text{m}$ (micrometer) per minute
 - Calculate the average growth for ALL measurements on EACH plate
 - The average growth rate for each plate will be graphed in your bar graph
- Minimum/ Maximum value for each plate
- Standard Deviation: See below for instructions
- P-Value (T-Test): See below for instructions
 - For your data to be considered statistically significant, the value must be BELOW 0.05.

Standard Deviation:

1. Select the cell to be used to input your standard deviation.
2. Insert > Function > Type “STDEV” into the search bar and highlight it in the box below.
3. Press ok and a new box will come up. The first box will be selected.
4. Click on the tiny box with the arrow to the right of the box. This will allow you to select your data.
5. Drag your cursor on ALL the data in the column from your growth rate values for one plate. Once you let go, your data will be surrounded by moving dashed lines.
6. Press enter and the range will show up in the first box. Select ok and your standard deviation value will appear in the cell.

T-TEST (P-Value)

1. Select the cell that will contain your value.
2. Insert > Function > Type in “TTEST” into the search bar and highlight it in the box below.
3. Press ok and a new box will come up. There will be a total of four boxes. The first box will be selected and say “array 1”. Click on the tiny box with the arrow to the right of the first box. This will allow you to select your data.
4. Your data for array 1 will be ALL of your growth rate data from ONE plate. Drag your cursor and select all the growth rate values for your control plate.
5. Press enter and place cursor in the box labeled “array 2”. Select the growth rate data for a treatment plate the same way that you did above in #3. Do this same comparison between all of your plates.
6. For “tails” type in “2”.
7. For “type” type in “3”.
8. Hit enter and your P-Value will be entered into the cell.

About the T-Test: This function returns the p-value of the t-test, which when p is subtracted from 100, the value returned is the level of confidence that the two columns of values are statistically different.

Making the Table and Graph

(For Microsoft Excel 2007 and later versions)

1. Create a new tab at the bottom left hand corner of your Excel sheet and name this tab “Graph”
2. Label individual cells across the page with the type of plates. For example, “Control, 20 um ADPS, etc...” These will serve as your columns.
3. Make the rows for these columns by labeling “Average Growth, STDEV, TTEST”.
4. When typing the names of your control plates, you can insert the “ μ ”, “ γ ” or “ β ” symbol by copying the symbol from a word document (Insert > symbol). Excel does not have a symbol function.
5. Now that the table is set, select all the data that is to be input into a specific column or row. Select the copy icon or right click the data and select copy. Right click the highlighted data again and select **“paste special”**. Select “ALL” when the paste special box comes up and click ok. Select the empty cells that the data will be put in and press paste. The results of step 1-4 should look similar to this:

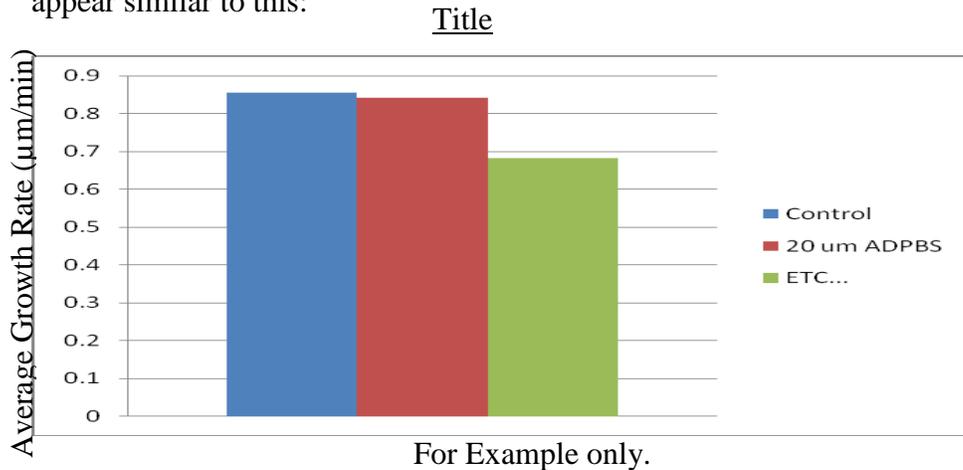
	<u>Control</u>	<u>20 um ADPS</u>	<u>Etc....</u>
<u>Average Growth</u>	0.855467517	0.840760656	0.84907323
<u>STDEV</u>	0.372602152	0.42741874	0.395164801
<u>TTEST</u>	N/A	0.863197143	0.85765575

IMPORTANT NOTE: When reporting your data please only go to three decimal points eg 0.043 or 1.238.

6. To make the graph, click the “insert” tab on the top left hand corner > choose “COLUMN” > under 2-D panel, select “CLUSTERED COLUMN.” An empty white box should open.
7. Go to the top left hand corner and choose “SELECT DATA.”
8. A data collection box should open. Click “ADD.” Another selection box should open and under “SERIES NAME” click the type of your plate (your column names created in step 2) Make sure that in the series name that you have inserted the right symbol, “ μ ”, “ γ ” or “ β ” by copying and pasting it from a word

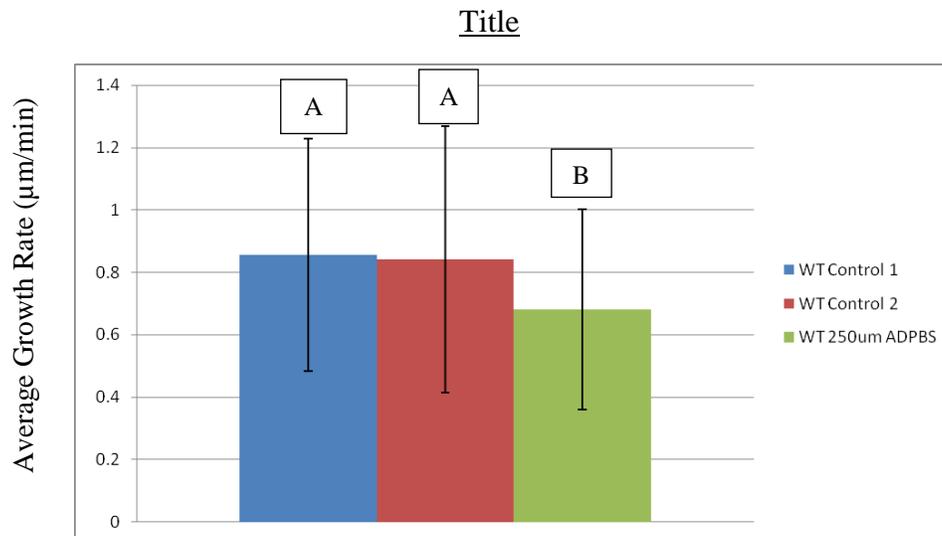
document. Note that both graphs below do not have the correct symbols inserted. For “SERIES VALUE” click the average growth for the corresponding plate type. Click “OK” and the box will close.

- Repeat step 7 for as many plates you have for your experiment. After seeing all your plate types are represented, click “OK.” A graph of multiple colors should appear similar to this:



- Inserting error bars must be done one at a time. Begin by selecting one of the color bars on the graph. Select the “LAYOUT” tab on the top right corner. Select “ERROR BAR.” A box should open select “MORE ERROR BARS OPTION” > click “CUSTOM” bubble > “SPECIFY VALUE.” A data collection box opens and for the + and – values, select the same standard deviation number for the corresponding plate type (Your standard deviation value that was calculated from your results). Repeat this step for every bar.
- For quick interpretation purposes, insert the correct letter in a text box and adjust it on top of each bar. The product of steps 1-10 should be similar to this:

For Example Only



Excel Notes:

1. When calculating a TTEST, normally "Array1" should be the control and "Array2" should be the treatment which you are comparing against the control. For tails, insert "2" and "3" for Type. 3 – two-sample unequal variance is correct for student t-test (Type 2 = Welch's t-test).
2. **Letters of Statistical Significance:**

In order to insert letters indicating statistical significance, create a textbox and type the letter and then position it above the standard deviation bar for each bar. Letters of significance are assigned based on a p-value of < 0.05 which indicates statistical significance. The p-value is obtained using the TTEST function in Excel which compares the average growth rate of one plate with the average growth rate of another plate and takes into consideration the standard deviations for each of those plates. First compare each treatment plate to the control plate and then compare the different treatment plates to each other. Letters are then assigned as follows: "a" is assigned to the control plate, "b" is assigned to the first bar which is statistically significantly different from the control plate and any other bars which are statistically significantly different from the control plate and statistically significantly the same as the other bars designated with a "b"; "c" would be assigned to a bar which is statistically different from the control plate as well as significantly different with another treatment plate.

Scientific Grammar:

- Make sure when typing chemical names into charts, presentation slides, etc... that you use the actual symbol to represent "beta", "gamma", "micro", etc. For example:
 1. Wrong: ADPBetaS, ADPBS
 2. Right: ADP β S
 1. Wrong: 30um, 30 micrometers
 2. Right: 30 μ m