Title	Leaf area measurement using ImageJ.	**Revised by:**	**GUA·QUYE Group.**
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Leaf area measurement using I*mageJ*

Table of Content

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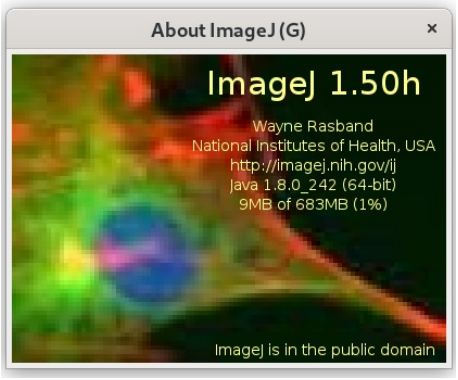
1 Introduction to ImageJ software

ImageJ is a public domain Java image processing and analysis program(“ImageJ Disclaimer,” 2018) designed with an open architecture to provide extensibility via Java plugins (Ferreira and Rasband, 2012). Being a public domain free software means that, every **ImageJ** user has the four essential freedoms defined by Richard Stallman in 1986: **0)** The freedom to run the program as you wish, for any purpose; **1)** The freedom to study how the program works, and change it to make it do what you wish; **2)** The freedom to redistribute copies so you can help others; **3)** The freedom to improve the program, and release your improvements to the public, for everyone's benefit (Free software foundation, 2019).



This guide is an adaptation (Reinking, 2007) to provide a specific example on how to measure leaf/needle areas of European beech (*Fagus sylvatica* L.) and Scots pine (*Pinus sylvestris* L.). In the example shown, the leaf area surface only considers one side of the leaf/needle (Nobel et al., 1993). The **ImageJ** version used is 1.50h and it is run under a Fedora GNU/Linux environment.

2 Software download

The software **ImageJ** (Schneider et al., 2012) is available for download on **GNU/Linux, MacOS, and Windows** platforms in the following link: https://imagej.nih.gov/ij/. Instructions are simple to

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follow and additional information on plugins, requirements, etc., are offered through the user guide (Ferreira and Rasband, 2012).

3 Samples used

For this guide, we have narrow two options available for **ImageJ** use: **i)** s**canning new material** and **ii) already scanned material.** Both options can be solved using this guide. Attached you will find two images included, for your use, as an option to control the results after following the instructions here presented. These images are attached into the **.ZIP** file and included in **.PNG** format.

4 Procedure

4.1 Scanning new material

The scanner should be set to a known paper size format to simplify the process. Image quality depends on resources and project requirements. Here, the scanner was set to **A4** (210 x 297 mm) and 75 px (our scanner lower resolution).

4.2 Already scanned material

In the case of material that has been previously scanned, please control the scale used so the measurements are reliable (Fig. 2). After you have identified the scale and made the corrections required, follow the measurement procedure here stated.

4.3 Measurements

The main procedure can be applied indistinctly to leaf or needle measurements of any specie. The following example is applied over 10 needles of *S.pine* and five leaves of *E.beech.*

1. Open and convert the scanned image:
To convert to gray scale, click on :

Image → Type → 8-bit

2. Setting the measurement scale:
Option1: Draw a line over a scaled ruler if present on the scanned image.
Option2: Draw a line (vertically) covering the length of the page size previously set on the scanner (in our case A4: 297 mm). Then, in the main menu click on:

Analyze **→ Set Scale**

In the *Set Scale window* displayed, enter the length used as reference into the "***Known Distance"*** box and write the used units in the *"**Unit of Measurement"*** box. Check the box ***"Global"***.

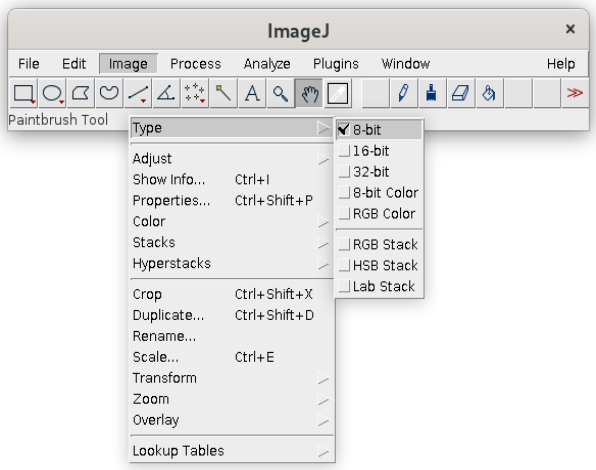

Fig 1: Initial steps.

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Fig 2: Setting the scale.

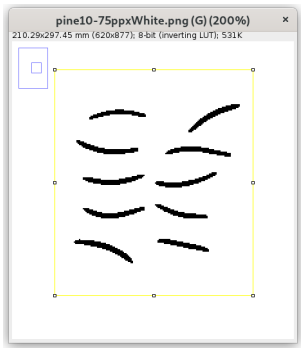


Fig. 3: Setting the threshold.

Confirm that the measurement scale is correct!, by drawing a line over the image (yellow line). If the whole page was used as reference, the scale also appears in the **upper left corner** of the output window (Fig.2.).

3. Threshold leaf/needle image:

Process → Binary → Make Binary

As result, the automated threshold should only include areas selected.

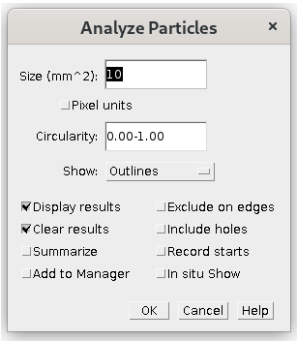
Enclose the leaf/needles with the rectangular selection (yellow rectangle) tool for the next step (Fig.3).

4. Calculate the area of the leaf/needles :

Analyze → Analyze Particles

Enter **10** as the minimum particle size (value for this specific case), mark **"Show Outlines"**, check **"Display results"** and click **"OK".**

The outline of every leaf/needle is automatically drawn and numbered (Fig.4).



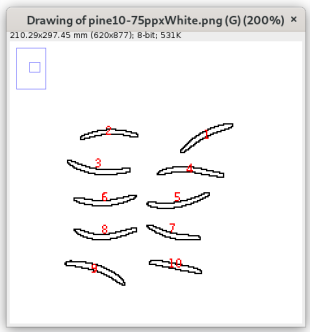
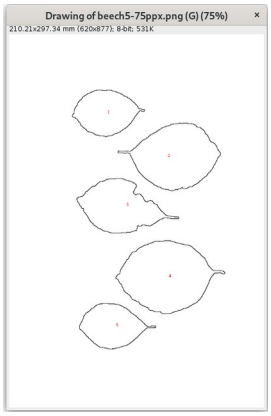


Fig. 4: Analysis *S.pine*



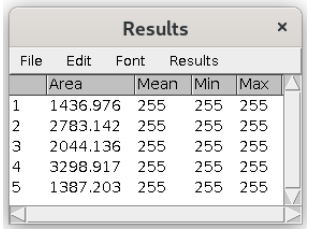


Fig. 5: Analysis *E.beech*

In the case of *E.beech* enter **50** as the minimum particle size, and follow the same procedure as with pine for the area calculation (Fig. 5).

The different choice for **particle size** remains on the personal needs as a reference value to determine the area calculation. Therefore, the small value (10 mm) in the case of *S.pine* needles (smaller than leaves) was selected.

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5. Results and data saving:

The results window gives an estimated area for each needle in mm^2 (for the example 20.592, 16.795, 21512, ...17.255) (Fig.6).

Additional information (mean, standard deviation, min, max, etc) can be also automatically customized and presented in the final results output (summary).

Finally, the results table can be saved as **.csv** or **.txt** file for further manipulation.

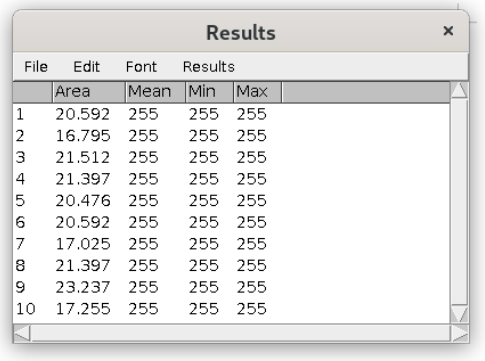

Fig. 6: Results output for *S.pine*

5 References

Ferreira, T., Rasband, W., 2012. ImageJ User Guide 198.

Free software foundation, 2019. The four essential freedoms [WWW Document]. What Free Softw. URL https://www.gnu.org/philosophy/free-sw.html#f1

ImageJ Disclaimer [WWW Document], 2018. URL https://imagej.nih.gov/ij/disclaimer.html

Nobel, P.S., Forseth, I.N., Long, S.P., 1993. Canopy structure and light interception, in: Photosynthesis and Production in a Changing Environment. A Filed an Laboratory Manual. Chapman & Hall, pp. 79–90.

Reinking, L., 2007. Examples of image analysis using ImageJ.

Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671–675. https://doi.org/10.1038/nmeth.2089

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