



Leaver-Toaster using

Goal : How to use the Leaver module from Toaster plugin

Note

The L-Toaster plug-in is composed from the three distinct tools: the **Leaves Analyzer**, the **Leaves with aids Analyzer**, and the **Education Leaves Analyzer**. It proposes **batch image processing tools**.

Contents

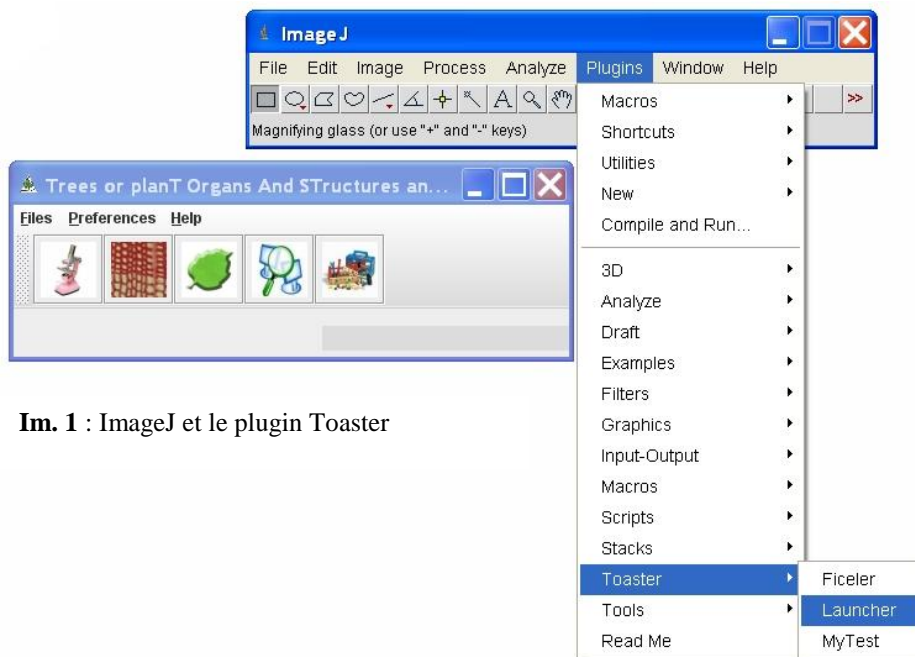
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1. Toaster running

The *Toaster* plug-in is run from the *ImageJ*¹ application. So,

- Run ImageJ...
- Select the *launcher* item of the *Toaster* sub menu of the *plugins* menu



Im. 1 : ImageJ et le plugin Toaster

2. Toaster setting

The *Toaster* plug-in manages the working folders and the export format: the user can specify where find input data or achieve output results,... and this once and for all.

- Select the *modify* item of the *Preferences* menu



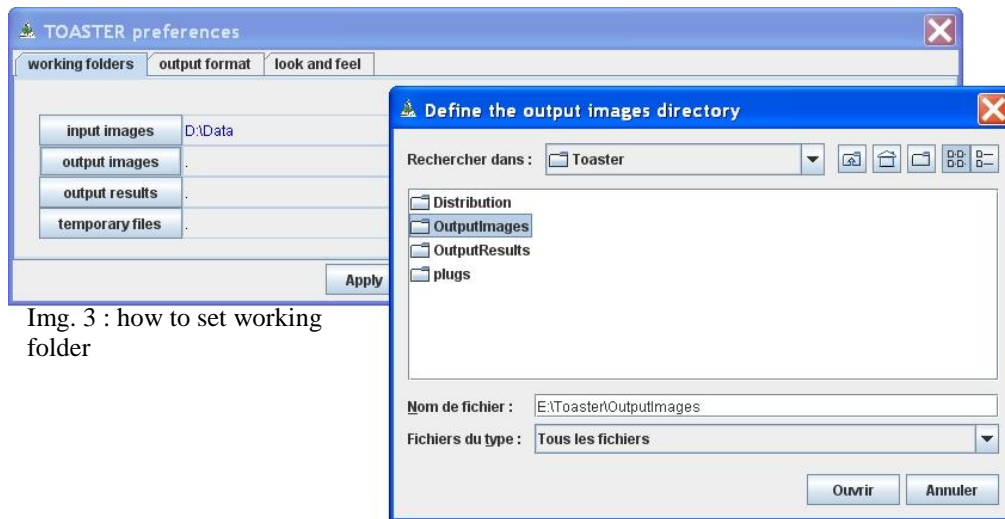
Img. 2: the folders setting

¹ **ImageJ** is an open-source software developed by the *National Center for Biotechnology Information* (NCBI), for the images processing and analysis. It is distributed under GPL licence.



2.1. The working folders

In the *working folders* page, set the different fields from the corresponding browsers (the buttons located at the left of the text fields) (see img 3.). **Save** the settings if you want the application uses it at each time; in other case, **Apply** only the settings (see img 2.).



Img. 3 : how to set working folder

Note: rules of colors (fig 4.)

Different colors are used to indicate the specified paths.

- ✓ In black: validate path already used – *the indicated folder exists and has been already used*
- ✓ In blue: path modified by the operator– *the indicated folder exists when it is selected from the browser*
- ✓ In red: invalidated path – *the indicated folder does not exist on the current computer; you should give a good path.*



Img. 4 : rules and colors to specify the working folders

The fields :

input images : folder in which the plug-in takes the images or the repertories containing the images to process.



output images : folder where the plug-in saves the results images, i.e. the visual outings used to check the processing.

output results : folder where the plug-in saves the results files, i.e. the numerical outings produced by the processing.

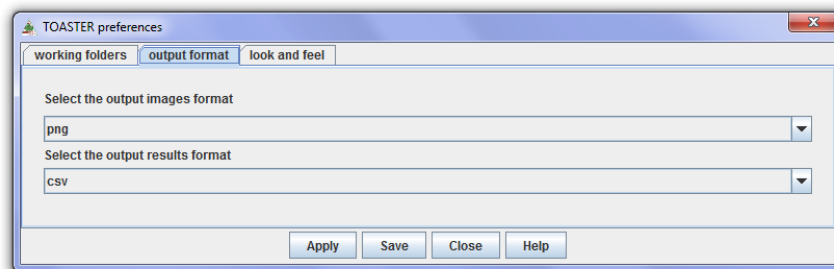
temporary files : folder where are achieved the temporary files.

Note: folder syntax (fig 4.)

The working folders are normally given by their extend name, that is to say from the root. However, a relative path can be displayed. The “.” means the local ImageJ folder, that is to say the repertory where is installed the ImageJ application.

2.2. The export formats

In the page *output format*, set the different fields using the drop-down list (*voir img 5.*). **Save** the settings if you want the application uses it at each time; in other case, **Apply** only the settings (see *Img. 5*).



Img. 5 : format des fichiers d'export

The fiels :

Images format: concerns the format of graphical results. Several graphical formats are available: bmp (*BitMaP*), gif (*Graphics Interface Format*), jpeg (*Joint Photographic Experts Group*), png (*Portable Network Graphics*) and tiff (*Tagged Image File Format*).

Results file format: concerns the format of numerical results. Several standard formats are available: txt (*TeXT*), csv (*Comma Separated Values*, loadable by the usual spreadsheets).



3. Data loading

Different modes are available: load (and process) **only one file**, or load (and process) **all the files of a folder**.

3.1. I want process only one file

Use the first submenu of the files menu... A browser is then opened and allows selecting THE file to process.



Note: results

The output data (image file or/and results file) will be saved into the output folders under the same root name as the selected file.

3.2. I want process several files

Use the second submenu of the files menu to select several files. A browser is then opened and allows selecting A file of the folder to process. *All the files of the folder where is the selected file will be processed.*



Note: results

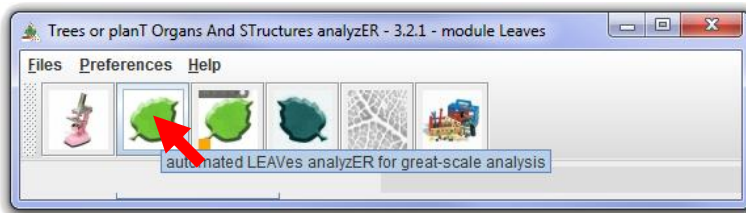
The output data (image file or/and results file) will be saved into under the same root name as the selected file, achieved in a subfolder created in the output folders.



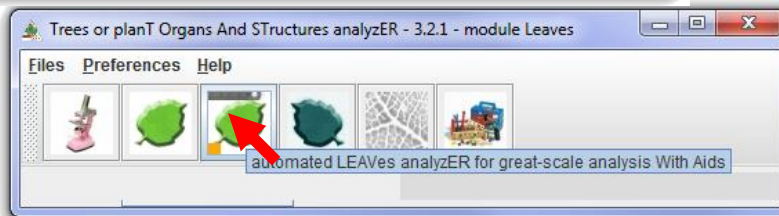
Warning: the input folder has to contain only image files produced from the same settings, with the same optical resolution (value generally given in dpi).

4. Leaver opening

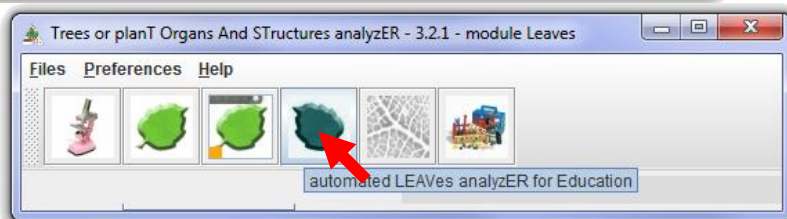
The Leaver plug-in is ran by the green leaf icon in the tools bar of Toaster



to process single images containing several leaves arranged by line



to process images containing several leaves arranged by line containing some additional aids (rule, stickers,...)



Derived version for Education: does not integrate the petioles deletion.

Note

- *At least one image must be loaded before the Leaver running.*
- *In the second case, the aids should be given on a numeric image produced according to the protocol applied for the leaves study; the produced image will be named "aids.jpg" and put in the folder of the images to process.*



5. Leaver setting

The following dialog box allows adapting the processing to the current (set of) image(s). Three aspects are concerned: the metrical aspect to define the significant object sizes, the processing aspect and the managing aspect giving the working folders and the export formats.

5.1. The Managing aspects

The managing aspects are set from the Preferences panel of the Toaster plug-in (see §2). Are editable the following fields:

Output images - path: extend name of the folder where will be achieved the output images.

This field is set from the Toaster settings. These images are built to avoid a visual checking after data treatment. The leaves are colored in regard to their position in the treated image: the hue depends on the line, and the saturation on the row in the line (§7).

Output results - path: extend name of the folder where will be achieved the output results.

This field is set from the Toaster settings. These files contain the numerical values of calculated parameters, sorted by natural reading order of leaves – from left to right, from up to down.

Output results - saving: the way to archive the results

- ✓ **Only one results file:** the results of all read input files are written in only one output file.

The output file takes the name of the input folder (when several files are processed) or of the input file (when only one image is treated). In the results file, each line corresponds to one leaf; its name is defined by the following way:

[image_name] , [number of line]_[row of leaf into the line].

- ✓ **One results file per image:** the results are saved image per image, in different files.

The output files follow the same organization as the input file. In each results file, each line corresponds to one leaf of the corresponding image; its name, called identifiant, is defined by the following way:

[number of line]_[row of leaf into the line]

Output images format: give the format of the output images

Output results format: give the format of the output numerical files

The csv files are loadable by the usual spreadsheet; the txt files can be imported in the usual spreadsheet.



5.2. The processing aspects

Internal - measures: defines which parameters (*see §8*) will be evaluated:

- ✓ **Default measures**: only the area, the length and the width of each found leaf will be returned ; *length and width are defined by the radius of the fitted ellipse*;
- ✓ **Use IJ measures setting**: all parameters selected into the “*set measurements*” dialog box of the ImageJ platform (*see analyse / set measurements* for more details) will be evaluated.

About petiole: defines the processing that will be applied

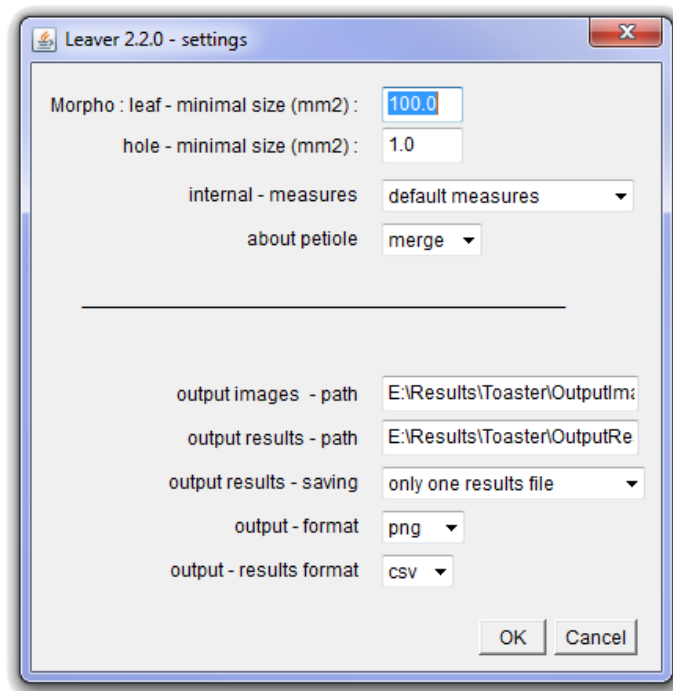
- ✓ **merge**: the petiole is merged to the blade; the results include the two parts.
- ✓ **delete**: the petiole is suppressed before computation; the results concern only the blade.

5.3. The metrical aspects

They avoid defining the sizes of the significant objects. Two cases are possible:

Case of TIFF images

Only two parameters are :





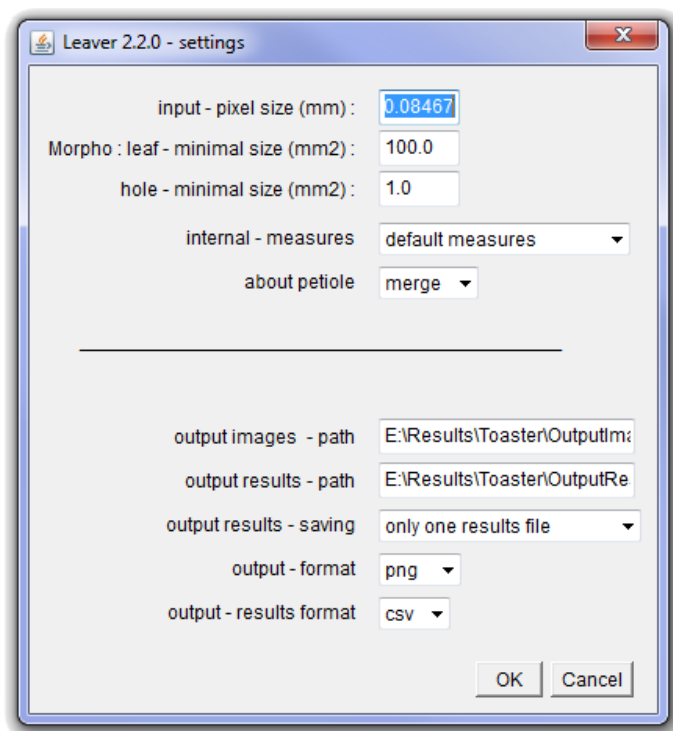
Morpho – leaf minimal size: this is the minimal size, given in squared millimeters, of significant object: all object having an area smallest than the given value will not consider as leaf and will be excluded from computation.

Morpho – hole minimal size: this is the minimal size, expressed in squared millimeters, of significant hole : all hole having an area smallest than the given value will be filled.

Scale factor: for the tiff images, the pixel scale factor is valuated from the optical resolution read in the header of the image.

Case of other images

Three fields are editable:



Input – pixel size: this is the scale factor, i.e. the size in millimeter of the pixel height (or width). This value is given subdividing 25,4 (the millimetric value of the inch) by the optical resolution of the image, given in dpi (dot per inch). The dpi is set for the digitalizing step, or read from an images editor such as Gimp².

² The GIMP is an freeware to create and manage images and numerical photography.



Morpho – leaf minimal size: this is the minimal size, given in squared millimeters, of significant object: all object having an area smallest than the given value will not consider as leaf and will be excluded from computation.

Morpho – hole minimal size: this is the minimal size, expressed in squared millimeters, of significant hole : all hole having an area smallest than the given value will be filled.

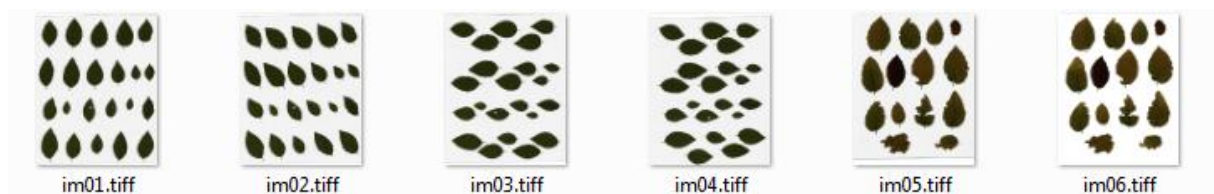
6. Leaver running

The Leaver plug-in is ran when you validate your settings: click on button “ok”.

7. Example

7.1. Data and results

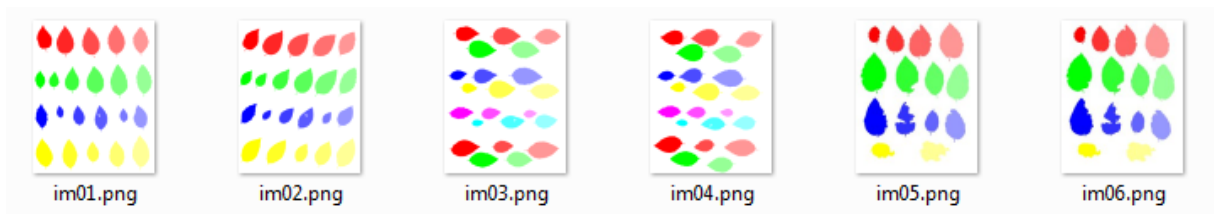
The input data are six tiff images located into the folder A317



NB: here, the images are produced by scanner: they are seen from under.

The settings of the §5 give the following output data:

- ✓ the checking images achieved in the folder `E:\\Toaster\\OutputImages\\A317`



NB: the images are reversed by vertical symmetry; so, they are seen from above, like their disposition on the scanner pane.

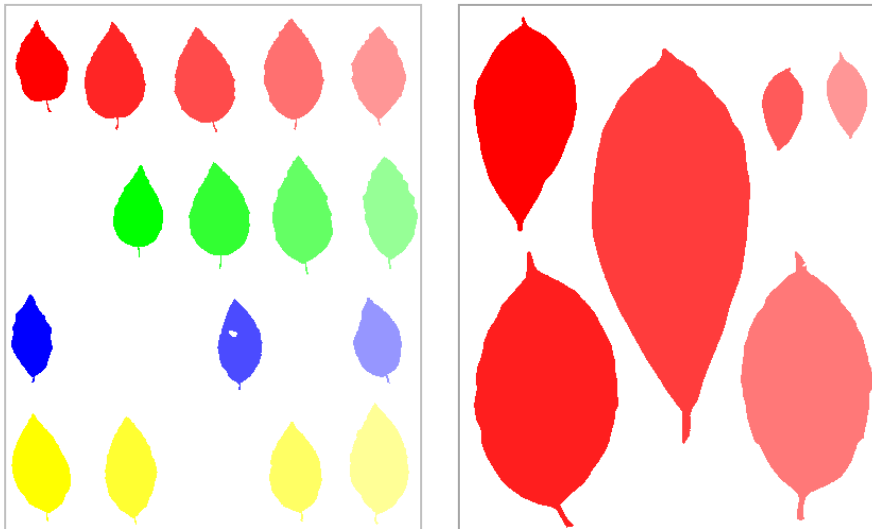
- ✓ One results file, named `A317.txt`, achieved in the folder `E:\\Toaster\\OutputResults`



7.2. Coherence checking

The coherence checking allows verifying the good ordering of results. For the large-scale analysis, it is important to check the weighing/areas coupling, without offset between weighing and geometrical measure.

The lines are drawn with different hues to ease the visual post-checking. The intensity depends on the row of the leaf in the line.

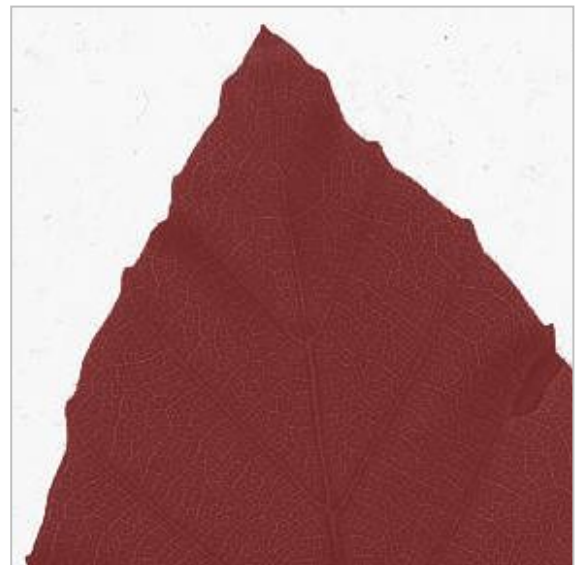


Im. 6: incoherence of the coloration, incoherence of the results. Left: good coloration of the result image, even if several leaves are missing. Right: only one hue showing for several lines; the results are not well sorted and could not be coupled with weighing.

7.3. Accuracy checking

The accuracy checking allows verifying the good segmentation of the images, i.e. the real accuracy of the leaf identification.

The checking images are produced by overlay of the segmented regions on the native images. The anatomical structures of each leaf are seen by transparency to well appreciate the segmentation quality.





7.4. Numerical results

The results file, called here *A317.csv*, is achieved in the *E:\\Toaster\\OutputResults* folder.

	A	B	C	D	E	F	
1	Image	Identifiant	Area	adjMajor	adjMinor	circ.	
2	im01	01_01	655.9054982	44.560833	24.851326	0.56561863	
3	im01	01_02	929.8547711	51.72825	29.467371	0.59902257	
4	im01	01_03	929.0088946	51.40731	29.663826	0.6211946	
5	im01	01_04	954.965832	53.280506	28.851784	0.62881607	
6	im01	01_05	764.1705146	47.916622	26.236246	0.6356188	
7	im01	02_01	319.4115475	32.10198	16.664772	0.60587	
8	im01	02_02	288.7807844	29.460972	16.8848	0.56780344	
9	im01	02_03	627.6188164	44.371452	23.767012	0.58225685	
10	im01	02_04	909.0591137	51.572212	29.208315	0.5969762	
11	im01	02_05	1012.170018	57.096497	28.637869	0.60268813	
12	im01	02_06	873.4391133	53.791866	25.43952	0.600011	
13	im01	03_01	513.2821275	42.303856	20.227884	0.5813066	
14	im01	03_02	160.3581022	21.741728	11.660619	0.6432554	
15	im01	03_03	420.2285502	34.264194	19.03625	0.6478899	
16	im01	03_04	576.7300293	43.486485	21.407778	0.6160087	
17	im01	03_05	205.935072	23.605568	13.621956	0.68553084	
18	im01	03_06	604.7012995	42.77645	22.971014	0.60379565	
19	im01	04_01	877.5394635	52.14375	27.245235	0.6232924	
20	im01	04_02	817.1166453	52.076164	25.114407	0.61350995	
21	im01	04_03	463.6334813	36.79185	20.23639	0.6205293	
22	im01	04_04	758.8085182	48.101654	25.112564	0.6115866	

This file is loadable in the usual spreadsheet, such as *Excel*.

NB: the numerical values are given in Anglo-Saxon notation

Each line corresponds to a processed leaf. The two first columns allow identifying the leaf in the images series: the first gives the image name, the second the line and the row of the leaf.

NB: When the checking images show incoherence, the numerical file will be modified to ease the results processing.



8. Measurements

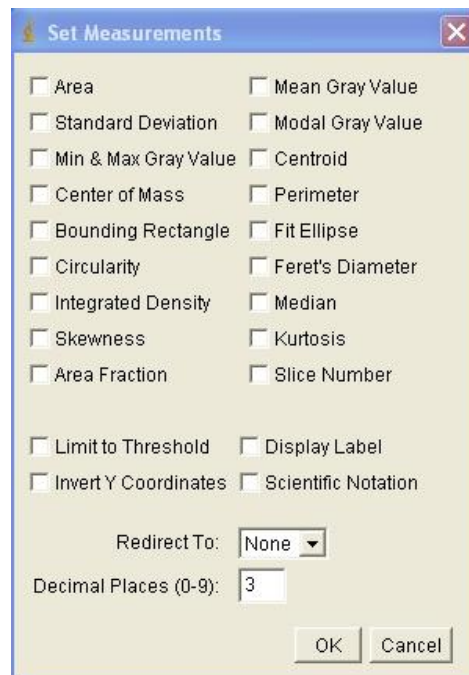
8.1. Default measurements

Area - Area of selection in square pixels. **Area** is in calibrated units, such as square millimeters, the calibration is made from the optical resolution given in dpi.

Adjusted length and width – they are defined by projection of every point of the leaf on the primary and secondary axes of the best fitting ellipse; the lengths are given in millimeter.

8.2. ImageJ Measurements setting...

Use this dialog box to specify which **measurements** are recorded by Analyze/Measure and Analyze/Analyze Particles.



Area - Area of selection in square pixels. **Area** is in calibrated units, such as square millimeters, if **Analyze>Set Scale** was used to spatially calibrate the image.

Mean Gray Value - Average gray value within the selection. This is the sum of the gray values of all the pixels in the selection divided by the number of pixels. Reported in calibrated units (e.g., optical density) if **Analyze>Calibrate** was used to calibrate the image. For RGB images, the mean is calculated by converting each pixel to grayscale using the formula $gray=0.299*red+0.587*green+0.114*blue$ if “**Weighted RGB Conversion**” is checked in **Edit>Options>Conversions**. or the formula $gray=(red+green+blue)/3$ if not checked.



Standard Deviation - Standard deviation of the gray values used to generate the mean gray value.

Modal Gray Value - Most frequently occurring gray value within the selection. It corresponds to the highest peak in the histogram.

Min & Max Gray Value - Minimum and maximum gray values within the selection.

Centroid - The center point of the selection. This is the average of the x and y coordinates of all of the pixels in the selection.

Center of Mass - This is the brightness-weighted average of the x and y coordinates all pixels in the selection.

Perimeter - The length of the outside boundary of the selection.

Bounding Rectangle – It is the smallest rectangle enclosing the selection. Uses the headings BX, BY, Width and Height, where BX and BY are the coordinates of the upper left corner of the rectangle.

Circularity - $4\pi(\text{area}/\text{perimeter}^2)$. A value of 1.0 indicates a perfect circle. As the value approaches 0.0, it indicates an increasingly elongated polygon. Values may not be valid for very small particles. For small particles, the circularity can be >1 (due to the definition of perimeter and area in a square grid). In those cases the value of 1.0 is returned (before v1.37 this was set as -1.0).

Fit Ellipse - Fit an ellipse to the selection. Uses the headings Major, Minor and Angle. Major and Minor are the primary and secondary axis of the best fitting ellipse (see §8.1).

Angle is the angle between the primary axis and a line parallel to the x-axis of the image. Note that ImageJ cannot calculate the major and minor axis lengths if Pixel Aspect Ratio in the Set Scale dialog is not 1.0.

Feret's Diameter - The longest distance between any two points along the selection boundary. Also known as the caliper length. The Feret's Diameter macro will draw the Feret's Diameter of the current selection on the image.

Integrated Density - The sum of the values of the pixels in the image or selection. This is equivalent to the product of Area and Mean Gray Value. The Dot Blot Analysis example demonstrates how to use this option to analyze a dot blot assay.

Median - The median value of the pixels in the image or selection.

Skewness - The third order moment about the mean. The documentation for the Moment Calculator plugin explains how to interpret spatial moments.

Kurtosis - The fourth order moment about the mean.

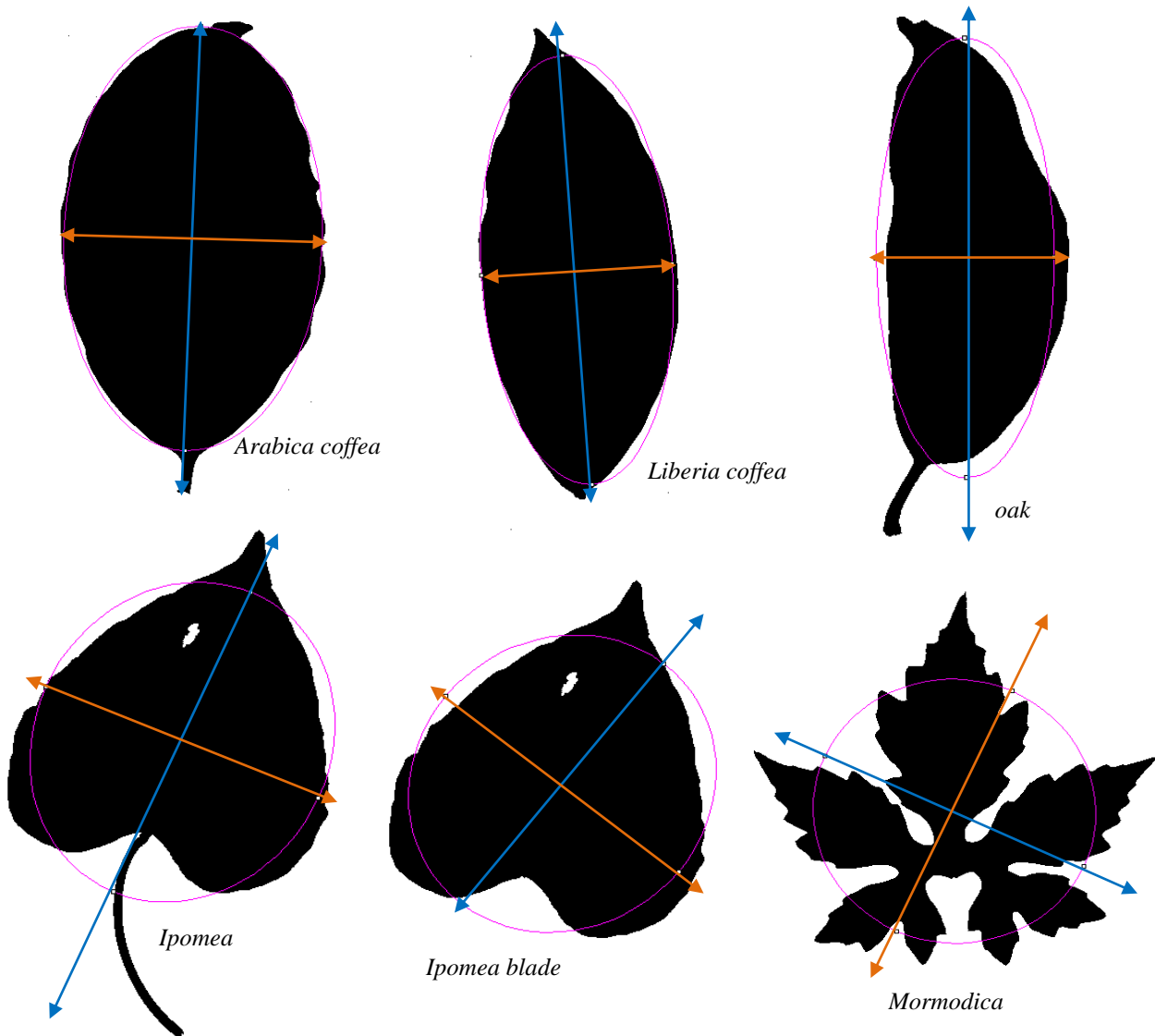


Area Fraction - The percentage of pixels in the image or selection that have been highlighted in red using Image/Adjust/Threshold. For non-thresholded images, the percentage of non-zero pixels.

8.3. About lengths

Lengths and widths must be used cautiously: they depend on the leaf shape, and on the applied method.

Without petiole deletion



Im 16 : leaves and lengths : in blue, the adjusted lengths, in orange the adjusted widths.



The lengths and the widths are here defined by projection of the leaf points on the primary and secondary axes of the best fitting ellipse; this approach is rather adapted to the single, elongated and right leaf.

With petiole deletion

To come

9. Reference

Borianne, P., 2012, *Automated valuation of leaves area for large-scale analysis needing data coupling or petioles deletion*, In Guo, Y., Kang, M. Z., Dumont, Y. Eds, Plant growth modeling, simulation, visualization and applications. Proceedings PMA12 : The Fourth International Symposium on Plant Growth Modeling, Simulation, Visualization and Applications, Shanghai, China, 31 October-3 November 2012, pp. 50-57

P. Borianne, M. Guérault, *Automated Evaluation of Homogeneous Leaves Area for great-scale analysis needing data coupling*, submitted to the international Conference on Image Processing Theory, Tools and Applications **IPTA'10, unpublished**