



## Surfol using

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**Goal :** How to use the Surfol module from Toaster plugin

### Content

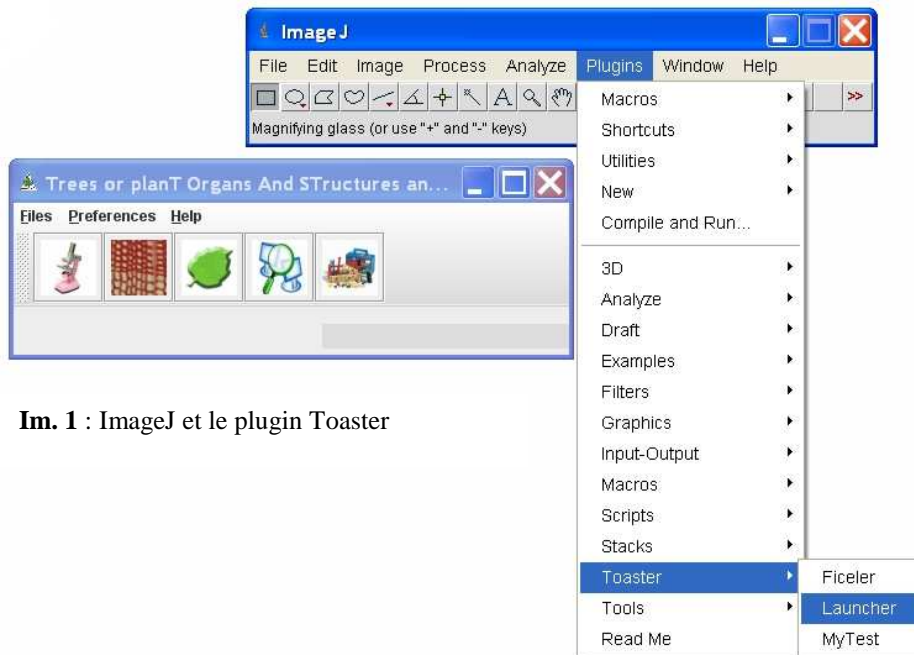
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## 1. Toaster running

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

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



Im. 1 : ImageJ et le plugin Toaster

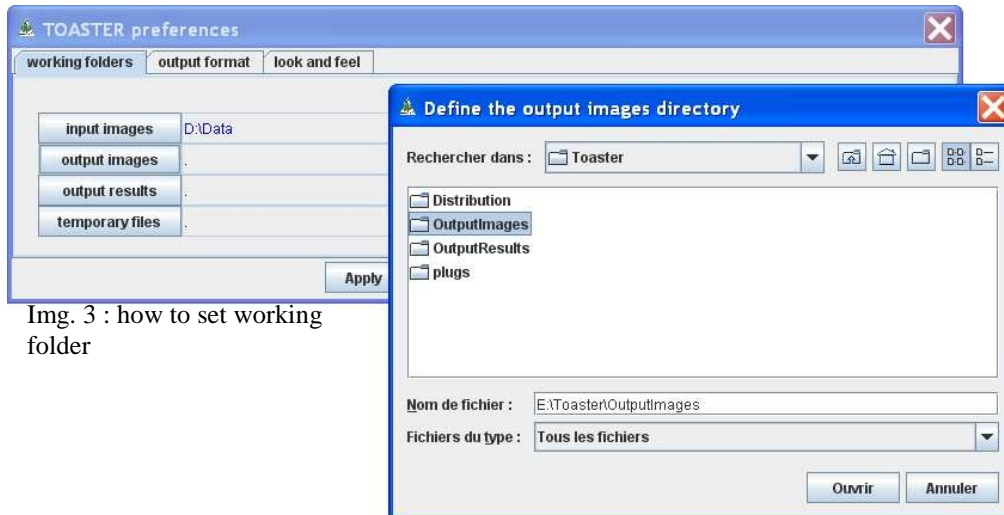
## 2. Toaster setting

The *Toaster* plug-in offers The working folders manager: 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
- 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 use it at each time; in other case, **Apply** only the settings (see img 2.).



Img. 2 : the folders setting



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

**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.



### 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...



**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, *in fact, all the files of the selected folder...*



**Note: results**

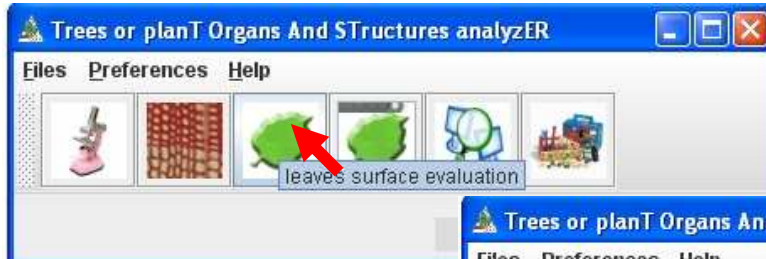
*The output data (image file or/and results file) will be saved into under the same root name as the selected file, achieved in a subfolder created in the output folders.*

**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. Surfol opening

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



Top: to process single images containing several leaves arranged by line

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

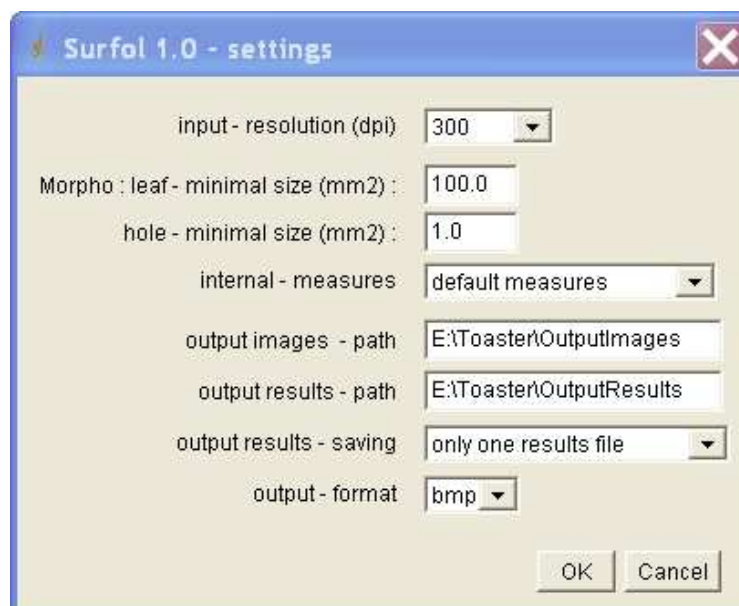


### Note

- *At least one image must be loaded before the surfol 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. Surfol setting

The following dialog box allows adapting the processing to the current (set of) image(s).





**Input – resolution:** this is the optical resolution, given in dot per inch (dpi); this parameter is fixed during the scanning step. This parameter can be given by some software as Photoshop® or TheGimp©. If you do not know this parameter, use the value “unknown” or “inconnue”.

### Note

*The optical resolution is used to convert punctual value in metric value. The results will be expressed in centimeter. When you choose the “unknown” value, the scale factor is fixed to 1: the pixels of the image are considered as unitary squares.*

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

**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.

**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 identifier, is defined by the following way:*

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

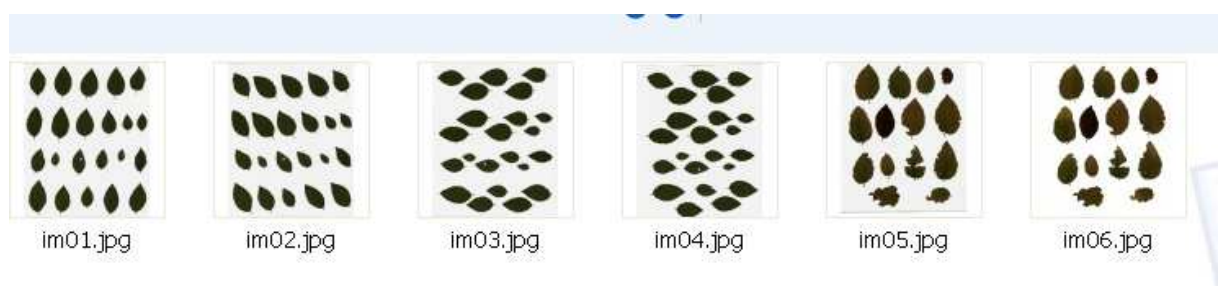
**Output format:** give the format of the output images

## 6. Surfol running

The Surfol plugin is ran when you validate your settings: click on button “ok”.

## 7. Exemple

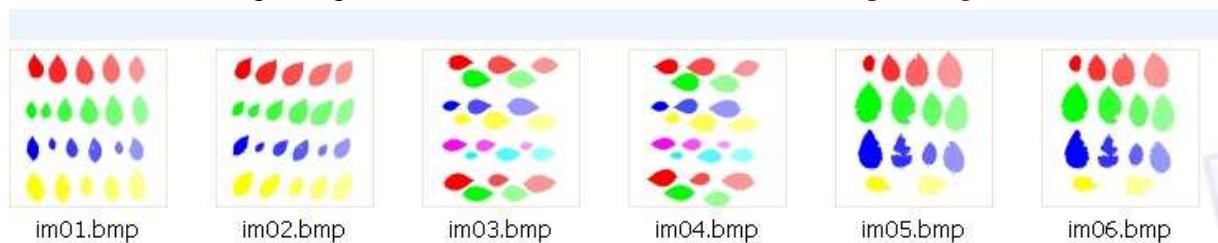
The input data are six jpeg images located into the folder A317



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

The settings of the §5 gives 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.*

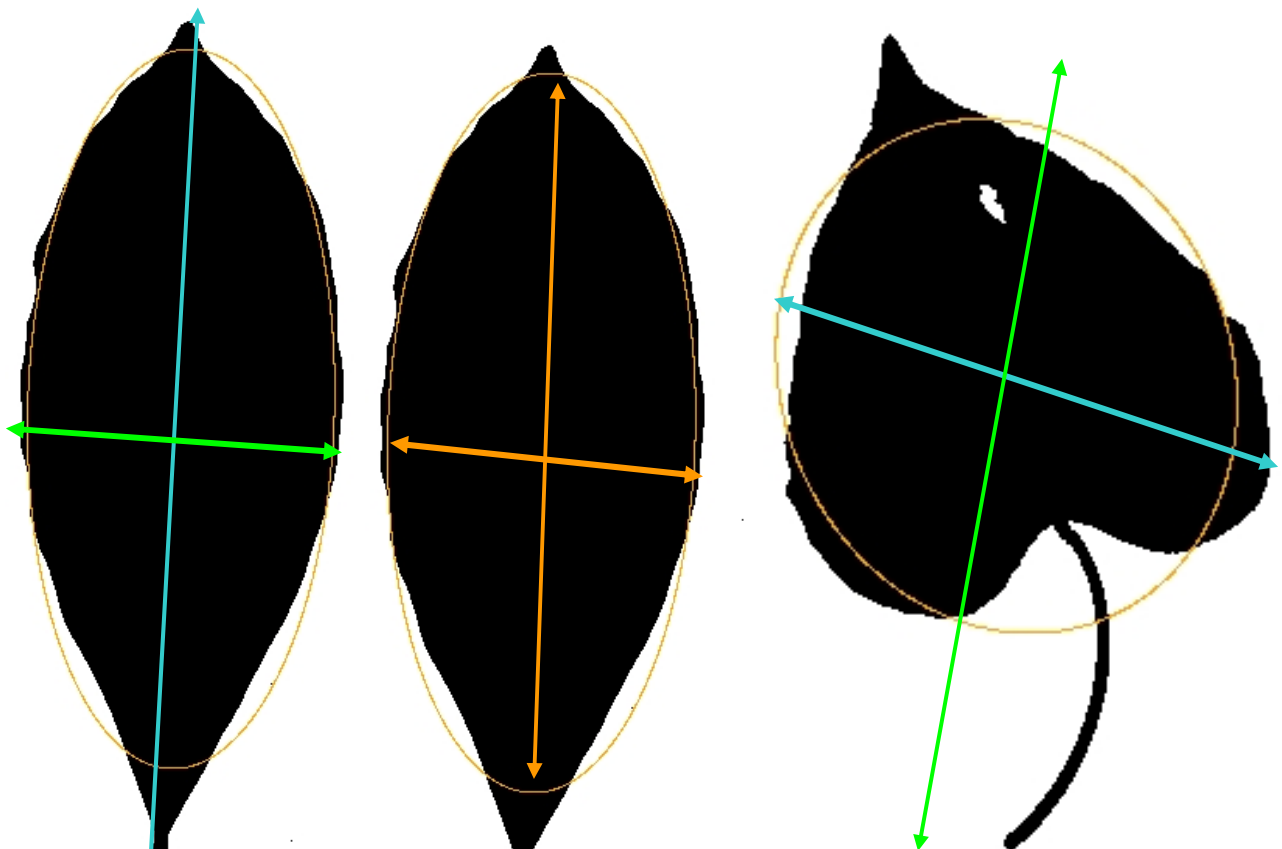


✓ One results file, named *A317.txt*, achieved in the folder *E:\\Toaster\\OutputResults*

```
A317.txt - Bloc-notes
Fichier  Edition  Format  Affichage  ?
Image ; Identifiant ; Area ; Major ; Minor ;
im01 ; 01_01 ; 655.9053949300219 ; 35.9601665410492 ; 23.223603884260257 ;
im01 ; 01_02 ; 929.8546246772947 ; 42.77545543312317 ; 27.677734759127198 ;
im01 ; 01_03 ; 929.0087483660592 ; 43.07463611431824 ; 27.460492222521975 ;
im01 ; 01_04 ; 954.965681611686 ; 45.03492715869507 ; 26.999047556707765 ;
im01 ; 01_05 ; 764.1703943248582 ; 40.147119946719364 ; 24.23516221525452 ;
im01 ; 02_01 ; 319.41149725452647 ; 26.14901165358765 ; 15.552686407621765 ;
im01 ; 02_02 ; 288.7807389671574 ; 23.79415816062378 ; 15.452829461545717 ;
im01 ; 02_03 ; 627.6187176068395 ; 35.638436850399174 ; 22.422670923237305 ;
im01 ; 02_04 ; 909.058970618868 ; 41.791458725121466 ; 27.69584734721924 ;
im01 ; 02_05 ; 1012.1698592698198 ; 47.472664474885256 ; 27.14687831492432 ;
im01 ; 02_06 ; 873.4389757838721 ; 46.37290740002197 ; 23.981611820180056 ;
im01 ; 03_01 ; 513.2820467237277 ; 35.79216647289734 ; 18.259050716820376 ;
im01 ; 03_02 ; 160.3580769689788 ; 18.761932126604922 ; 10.88236719528534 ;
```

## 8. Measurements

### 8.1. Default measurements



On left, adjusted length (in blue) and width (in green) on *Coffea arabica* : the petiole is included in the values which are over-evaluated.

On centre, the lengths given by ImageJ.

On right, the limits of the method, illustrated with *Ipomoea*: The axes of the best fitting ellipse do not follow the leaf orientation: in the case, the width will be greater than the length.



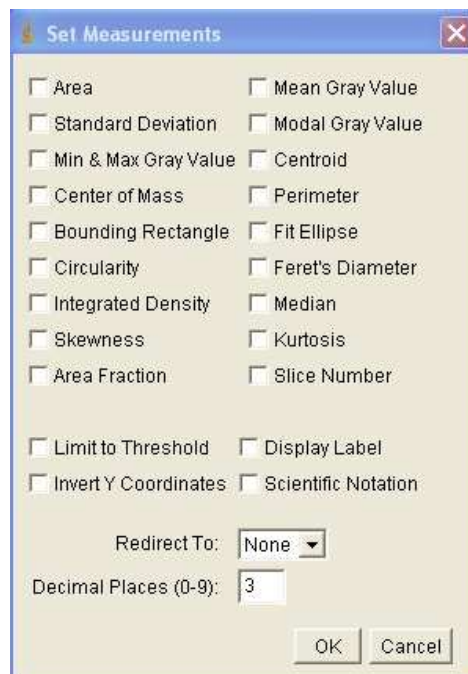


**Area** - Area of selection in square pixels. **Area** is in calibrated units, such as square millimeters, the calibration is made from the optical resolution given in dpi.

**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  $\text{gray} = 0.299 * \text{red} + 0.587 * \text{green} + 0.114 * \text{blue}$  if “**Weighted RGB Conversion**” is checked in **Edit>Options>Conversions**. or the formula  $\text{gray} = (\text{red} + \text{green} + \text{blue}) / 3$  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.

**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.

**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).

**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.

## 9. Reference

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**