Cefiler using

Goal : How to set and use the Cefiler module from plugins *Toaster* and *C-Toaster*.

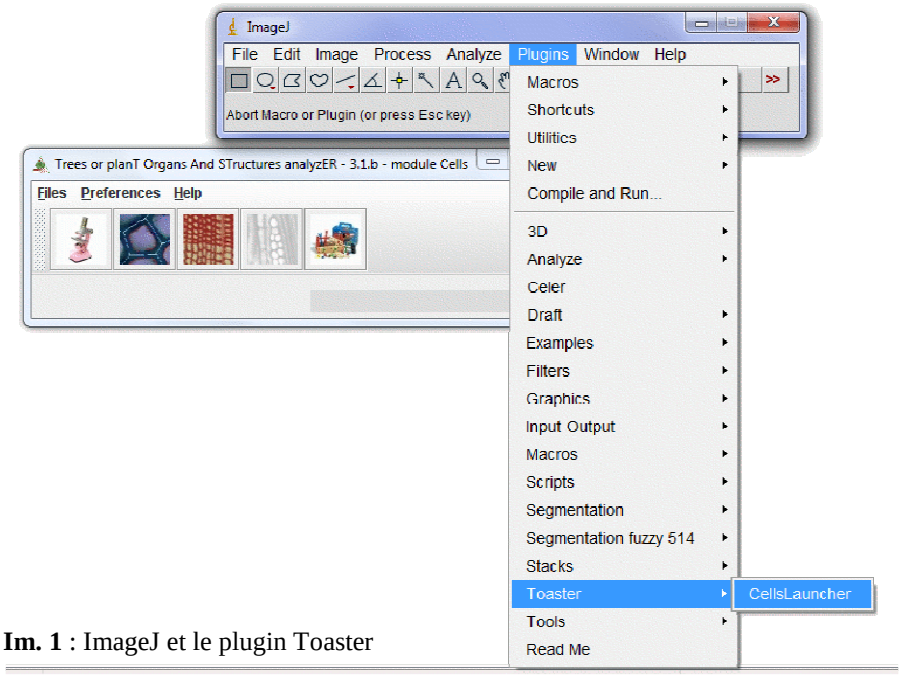
If you use **the basic IJ plugin**, i.*e. if you don't use the plugin Toaster*, **jump to the section 5**.

Content

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

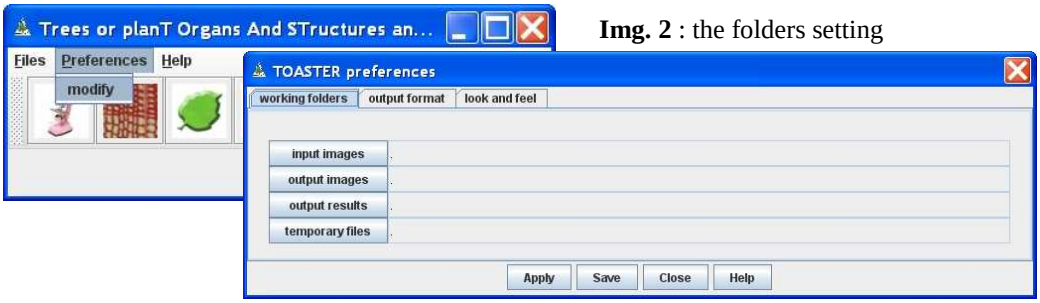


Im. 1 : ImageJ et le plugin Toaster

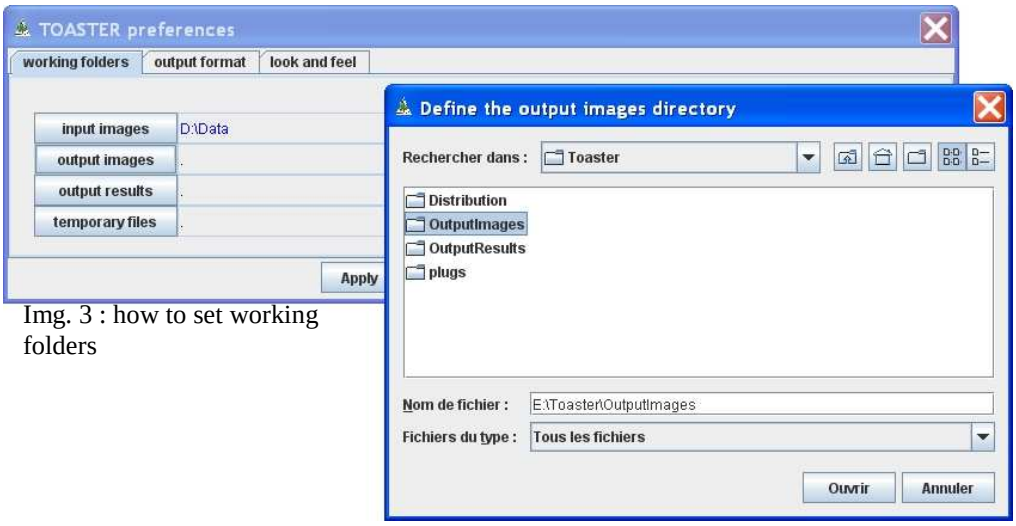
2. Toaster setting

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.).



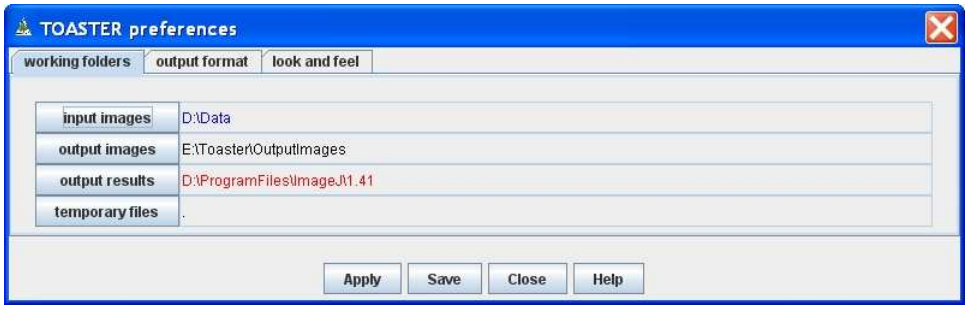
Img. 2 : the folders setting



Img. 3 : how to set working folders

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

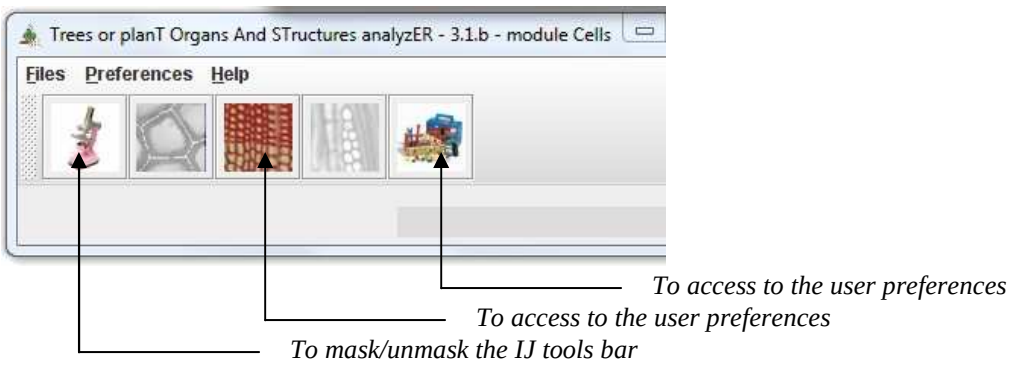
Use the second submenu of the files menu to select several files, *in fact, all the files of the selected folder…*

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. Cefiler opening

The plug-in cefiler is ran by the red cell icon in the tools bar of Toaster or C-Toaster

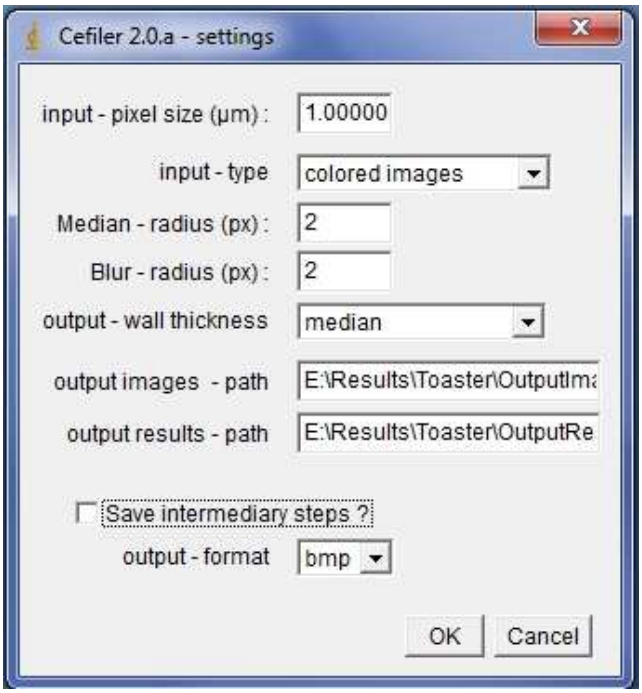


Note

- *At least one image must be loaded before the cefiler running.*

5. Cefiler setting

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



<u>Input - pixel size</u>: defines the size of the pixel length (or width) in micrometer. This value will be given by the user; it depends on the optical components of the used microscope to shoot the image. This value will be used evaluate the real dimensions of the cells.
The default value is 1.0: in this case, the returned dimension are expressed in pixels (or squared pixels).

Input - type: gives the type of the input images: colored or not colored… *For not colored images, one step is added to process.*

The default type is "colored images" ; the other case covers the raw or pumiced wood.

Median radius: defines the spatial neighborhood size used by the median filter. This value is given in pixels and represents the half size of the applied squared mask.

Note
This parameter cannot be defined automatically from the size of the images or from the cell dimensions. The given default value has been experimentally found. 2 seems the suitable value for all images.

Blur radius: defines the spatial neighborhood size used by the blur filter. This value is given in pixels and represents the half size of the squared mask applied to compute the mid value.

Note
This parameter cannot be defined automatically from the size of the images or from the cell dimensions. The given default value has been experimentally found. 2 seems the suitable value for all images.

Output – wall thickness: allow to choose the way to describe the wall thickness. Three options are available: the median value, the average and the standard, and all the values of the perimeter of the cell.

Output images - path: extended 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.

Output results - path: extended 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.

Save intermediary steps: Boolean indicating which images will be saved.

Note
The intermediary steps are the filtering, the watershed, the graph definition… they allow checking the well processing of the native images.

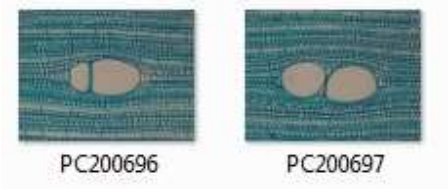
Output format: give the format of the output images

6. Cefiler running

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

7. Exemple

The input data are two jpeg images of broad-leaved trees located into the *Picnanthus* folder



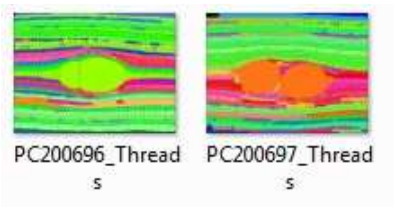
The settings of the §5 give the following output data:

7.1. Results location

The result images are achieved in the folder E:\\Results\Toaster\OutputImages\Picnanthus, and the result files in the folder E:\\Results\Toaster\OutputResults\Picnanthus

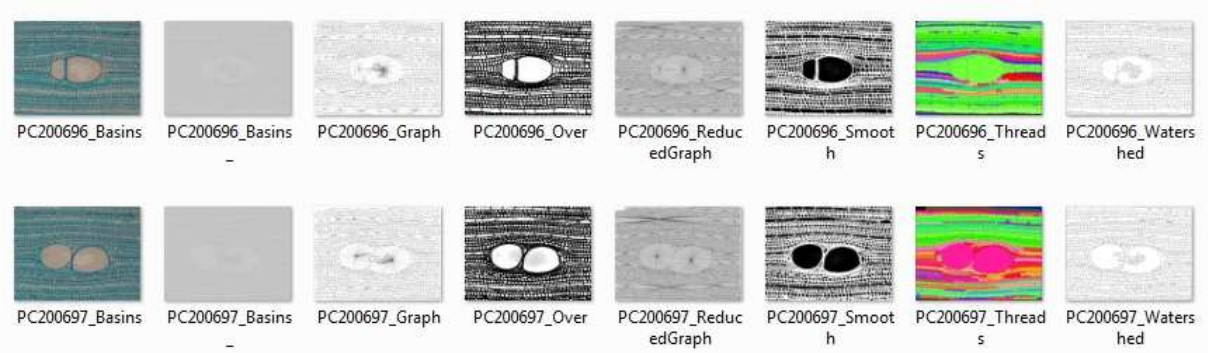
7.2. Output images

- If the “Save intermediary step” Boolean if off, only the final results are saving



Here, only the cell files representation is achieved

- If the "Save intermediary step" Boolean if on, all intermediary results are saving



Here, every processing step is achieved: smooth, watershed, basins, adjacency graph,…

- **The display rules**
  The cell files are displayed with the following coloration rules:
  - ✓ in **green**, the **continuous files**: these files are well-identified as *crossing the image*
  - ✓ in **blue**, the **dotted files**: the well-identified sub-files are linked by topological relationships,
  - ✓ in **red**, the **undefined files**: these are linear structures which need expert decision.

7.3. Output results

At each input image corresponds a CVS file, named as the input data: *PC200696.csv*, *PC200697.csv*,... Each file contains a summary description of the cell files. These values will be used by the *Cell Files Explorer*, developing software.

A	B	C	D	E	F	G	H	I	J	K	L	M	N
file	status	cell	xcenter	ycenter	perimeter	circularity	lumen_area	lumen_peri	lumen_circu	wall_area	wall_thickness (average)	wall_thickness (deviation)	
85	VERIFIED	788	947.0	427.0	80.04163056	0.743390864	114.0	44.38477631	0.727187900	265.0	4.628453890410957	1.2670167107968873	
85	VERIFIED	789	974.0	429.0	78.76955262	0.775696586	127.0	41.79898987	0.913444177	256.0	4.802403382352942	1.1766455773196707	
85	VERIFIED	799	996.0	430.0	63.45584412	0.814530066	53.0	27.55634918	0.877086395	208.0	5.019252714285713	0.7838473650303289	
85	VERIFIED	795	1015.0	430.0	62.38477631	0.784619131	79.0	32.97056274	0.913238549	164.0	4.541319499999999	1.6529020454441965	
85	VERIFIED	785	844.0	423.0	38.04163056	0.885710490	0.0	-1.0	-1.0	102.0	-1.0	-1.0	
85	VERIFIED	784	813.0	425.0	71.25483399	0.819236309	78.0	33.21320343	0.888552177	253.0	5.545422327586206	2.6280982229140153	
85	VERIFIED	783	868.0	423.0	64.42640687	0.717514014	25.0	19.31370849	0.842206381	212.0	6.0073015192307695	1.6881079265340062	
275	UNDEFINED	1019	510.0	533.0	49.69848480	0.742807744	0.0	-1.0	-1.0	146.0	-1.0	-1.0	
275	UNDEFINED	1013	530.0	530.0	77.59797974	0.525907755	0.0	-1.0	-1.0	252.0	-1.0	-1.0	
275	UNDEFINED	1011	551.0	530.0	88.32590180	0.413968053	0.0	-1.0	-1.0	257.0	-1.0	-1.0	
275	UNDEFINED	1009	570.0	529.0	55.59797974	0.617924521	0.0	-1.0	-1.0	152.0	-1.0	-1.0	
275	UNDEFINED	969	595.0	524.0	105.8822509	0.441631207	0.0	-1.0	-1.0	394.0	-1.0	-1.0	
275	UNDEFINED	939	631.0	512.0	108.5685424	0.761202245	16.0	20.48528137	0.449176737	698.0	12.725531085106383	5.101768963487581	
275	UNDEFINED	942	658.0	507.0	70.76955262	0.742691878	28.0	20.72792206	0.818948065	268.0	7.270743933333331	2.5007270238246972	
272	UNDEFINED	534	655.0	272.0	196.9533188	0.322334163	219.0	115.5979797	0.205946061	776.0	8.35041490340909	7.454745240853301	
272	UNDEFINED	583	699.0	281.0	40.04163056	0.650525683	0.0	-1.0	-1.0	83.0	-1.0	-1.0	
271	GOOD	263	83.0	128.0	400.1665222	0.339715954	3127.0	364.2670273	0.296140647	1202.0	3.3400684675675767	1.1581451385845172	
271	GOOD	264	233.0	129.0	337.9238815	0.414761304	2717.0	302.7106781	0.372601003	1052.0	3.4892485659164065	1.1153811129534235	
271	GOOD	254	376.0	126.0	384.1076477	0.362583140	3096.0	330.7106781	0.355724993	1161.0	3.618984049132957	1.4955286608487375	
271	GOOD	258	496.0	126.0	246.8528137	0.493693996	1636.0	207.1543289	0.479076796	758.0	3.800905310810813	1.7448045694145546	
271	GOOD	262	583.0	131.0	258.8528137	0.506557404	1879.0	216.3259018	0.504567877	822.0	3.837060017094018	1.5380151104364006	
271	GOOD	270	705.0	138.0	386.7939392	0.386626411	3277.0	348.7523086	0.338572845	1326.0	3.8583826742209717	1.3053464824895393	
271	GOOD	272	827.0	149.0	252.8528137	0.536386474	1876.0	207.7401153	0.546263220	853.0	4.111216324561402	1.7555079331311425	

file: file name – each file is identified by its own index

status: is linked to the relevance coefficient of the file identification; the coefficient is evaluated in regard to the methods used to recognize and join the files. This value is converted in the three following status:

- **Good** – the file is unambiguous identified ;
- **Verified** – the file is identified as a dotted line; several steps has been used, increasing the mistake risk ;
- **Undefined** – the recognized file present some ambiguities needing the expert advices.

cell: cell name – each cell is identified by its own index.

xcenter, ycenter: coordinates of the geometric center of the cell ; this is the average of the x and y coordinates of all the pixels of the cell, given in µm.

perimeter: length in µm of the cell border.

circularity: coefficient of circularity of the cell. It is given by the following by the following expression: 4π*area/perimeter^2. A value of 1.0 indicates a perfect circle. As the value approaches 0.0, it indicates an increasingly elongated shape

lumen_area: surface in squared µm of the lumen.

lumen_perimeter: length in µm of the lumen border.

lumen_circularity: coefficient of circularity of the cell. *See above for more details*

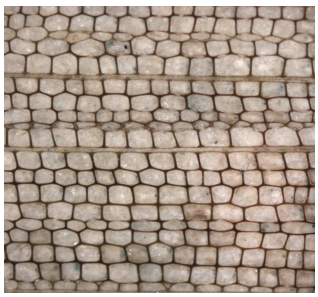
wall_area: surface in squared µm of the wall. *The cell area is implicitly defined by the sum of the both evaluated areas.*

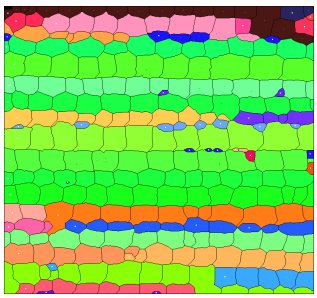
wall_thickness: three possibilities in regard of the dialog box setting : the median value, the average and the standard deviation, or all the distances of the wall border points to the lumen border. *In all cases, the values are given in µm.*

7.4. Results variability

Application to the raw wood

The processing of raw pumiced wood is possible under some conditions.
In the dialog box, select "non colored images" as input type.





Main limits

The transition between summer and winter wood is the main issue. The color properties between walls and lumina are reversed, the cell detection is very bad, as shown on the opposite side (*Albies Alba* – O. martin 2010).

8. Reference

Current work:

To come

Previous work:

P. Kennel, G. Subsol, M. Guéroult and P. Borianne, *Automatic Identification of Cell Files in Light Microscopic Images of Conifer Wood*, Image Processing Theory, Tools and Applications, IEEE IPTA'10, Djemal-Deriche Eds, ISBN 978-1-4244-7249-9, *pages 98-103* the international Conference on Image Processing Theory, Tools and Applications **IPTA'10**