Cell Analyzer ImageJ plug-in using

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Goal : How to use the Celer ImageJ plug-in

Celer is a half-automated plug-in used to analyze the geometry of cells (lumen size, wall size and thickness,…) provided by light microscopic images. The study processes only the sharp parts of the image: these parts are automatically detected by the application. The numerical results are saved in csv files, easily readable on spreadsheet.

Note
- ✓ The cell sharpness is evaluated by the positive difference between the mean lumen density and the mean wall density.
- ✓ The cell clustering, i.e. the distribution of cell points between lumen and wall is automatically done by k-means.
- ✓ Celer does **not allow batch image processing**. To process automatically several images, use the **Toaster application**
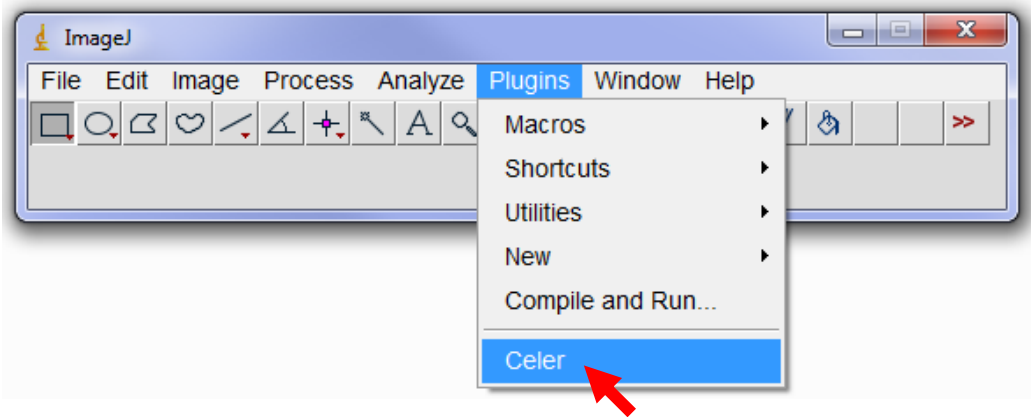
Contents

The Celer plug-in is opened from the Plugins menu of ImageJ

Note
- *One image must be loaded before to open Celer plug-in.*

1. Celer settings

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.
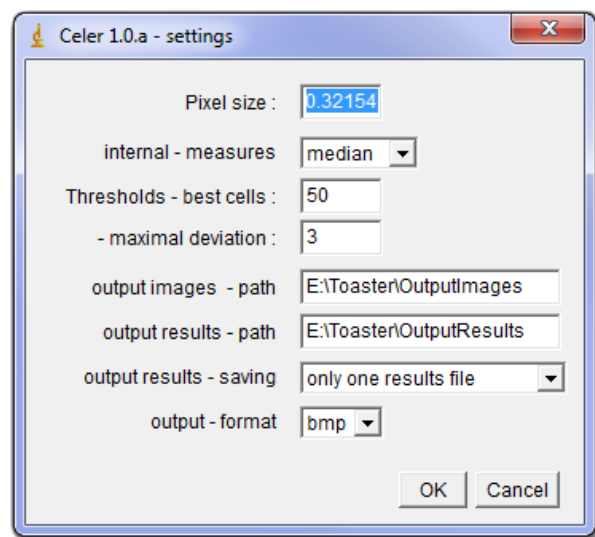
1.1. The metrical aspect

Input – pixel size: this is the scale factor, i.e. the size in millimeter (or micrometer) of the pixel height (or width). This value is by example given subdividing 25,4 (the millimeter 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 Gimp[1].*

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[1] The GIMP is an freeware to create and manage images and numerical photography.

1.2. The processing aspects

Internal - measures: defines which value (*see §8)* will be used to define the representative wall thickness. Default value is median thickness to not take account of outliers. The user can choose between different values: mean, minimal, maximal or median value, or the set of thicknesses evaluated from the wall border.

Note:
 ✓ The thicknesses are defined by a distance map between the two sides of the cell wall.
 ✓ If the *all* choice is selected, all the values are edited in the results file.

Threshold – best cells: gives the number of cells which will be considered in the results file.

Threshold – maximal deviation: the maximal value of the standard deviation of the thickness distribution of the cell wall. This is the threshold for acceptability.

1.3. 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 image processing.

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 – without effect the batch image processing being unavailable in this version

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.
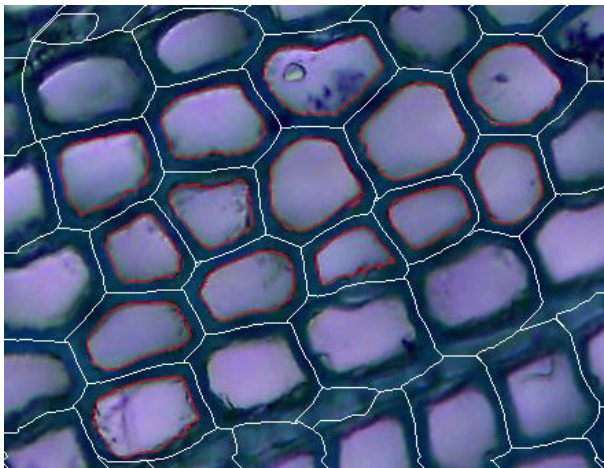
2. Celer running

The Celer plug-in is ran when you validate your settings: click on button "ok".

3. Example

The processing produces two types of results: a visual support to check the image segmentation, and numeric values characterizing the geometry of the best cells.

3.1. Focus on visual results

The cell boundaries are displayed in white, the lumen boundaries in red. The wall is defined as the space between the white and the red curves.

This checking image allows to verify the accuracy of the image segmentation.

3.2. Focus on numeric results

The results file is named like the input image, and is achieved in the given output results folder *(here E:\\Toaster\OutputResults)*

	Image	Sharpness	cell Area	Wall Area	Lumen Area	Thickness (med)	
1	Image	Sharpness	cell Area	Wall Area	Lumen Area	Thickness (med)	
2	test1	155.0976124	378.7101399	153.5311378	225.1790021	2.461984192	
3	test1	154.1807181	618.0532942	226.7298217	391.3234725	2.728356288	
4	test1	152.2510463	560.1560301	219.3892757	340.7667543	2.838692096	
5	test1	149.6493848	537.9276162	239.7567061	298.1709100	3.160232096	
6	test1	145.5419178	374.2644571	173.3816283	200.8828288	2.89386	
7	test1	143.4813602	466.0729759	206.4657792	259.6071966	2.89386	
8	test1	140.6434420	559.0187624	241.6176896	317.4010728	2.971878144	
9	test1	138.9720433	335.8041317	146.2939798	189.5101519	2.461984192	
10	test1	136.5876530	485.8200785	217.6316802	268.1883983	3.027046048	
11	test1	136.0074624	541.5461952	206.8793311	334.6668640	2.705506048	
12	test1	134.0443947	521.0753768	227.3501495	293.7252273	2.89386	
13	test1	132.8683784	406.2113404	184.6509172	221.5604231	2.595170240	
14	test1	129.5727200	308.1995433	158.9073123	149.2922309	2.57232	

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Celer Using – v.1.0.a

The results are sorted for each image by increasing cell sharpness (§4.). Here, the thickness is represented by the median value. The areas are given in squared units (millimeters or micrometers according to the user settings)

	A	B	C	D	E	F	G	H	I	J	K	L
1	Sharpness	cell Area	Wall Area	Lumen Area	Thickness measurements....							
2	155.0976124	378.7101399	153.5311378	225.1790021	1.6077	1.685718144	1.740886048	1.740886048	1.740886048	1.740886048	1.818904192	1.81890
3	154.1807181	618.0532942	226.7298217	391.3234725	1.6077	1.6077	1.6077	1.6077	1.685718144	1.685718144	1.740886048	1.74088
4	152.2510463	560.1560301	219.3892757	340.7667543	1.818904192	1.92924	2.007258144	2.062426048	2.062426048	2.140444192	2.140444192	2.14044
5	149.6493848	537.9276162	239.7567061	298.1709100	1.92924	1.92924	1.92924	1.92924	1.92924	1.92924	2.062426048	2.06242
6	145.5419178	374.2644571	173.3816283	200.8828288	1.818904192	2.007258144	2.007258144	2.140444192	2.140444192	2.140444192	2.140444192	2.14044
7	143.4813602	466.0729759	206.4657792	259.6071966	2.062426048	2.062426048	2.062426048	2.140444192	2.140444192	2.140444192	2.195612096	2.19561
8	140.6434420	559.0187624	241.6176896	317.4010728	2.383966048	2.383966048	2.461984192	2.461984192	2.517152096	2.517152096	2.517152096	2.51715
9	138.9720433	335.8041317	146.2939798	189.5101519	1.552532096	1.552532096	1.552532096	1.6077	1.6077	1.6077	1.685718144	1.68571
10	136.5876530	485.8200785	217.6316802	268.1883983	1.874072096	1.92924	1.92924	1.92924	1.92924	2.007258144	2.007258144	2.06242
11	136.0074624	541.5461952	206.8793311	334.6668640	1.552532096	1.6077	1.6077	1.6077	1.6077	1.6077	1.740886048	1.74088

Here, all the thicknesses are given for each "good" cell of the image. These values can study the distribution of the thicknesses for each cell wall.

4. Reference

To come