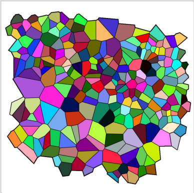

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Voronoi tesselation

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1 *Works for me* Share

dx.doi.org/10.17504/protocols.io.yxmvmnrx6g3p/v1

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ABSTRACT

This protocol describes how to perform Voronoi tesselation analysis of cerebellar images. It can be used for any biological images to study cellular sociology and is based on a model of parametrization and quantitation of
cellular population topographies developed by Marcelpoil and Usson (1992). It is advantageous to analyze cellular migration and dispersion in longitudinal studies.

DOI

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PROTOCOL CITATION

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MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

Marcelpoil, R.; Usson, Y. (1992) Methods for the study of cellular sociology: Voronoi diagrams and parametrization of the spatial relationships. Journal of Theoretical Biology **154**, 359-369.

KEYWORDS

Brain slices, Cellular sociology, Cell migration, Cell dispersion

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67460

GUIDELINES

N/A

MATERIALS TEXT

Voronoi Diagram Generator
by Frederik Brasz

FIJI (Image J)
by NIH

Microsoft 2013 and above
Windows 10
by Microsoft

SAFETY WARNINGS

None

DISCLAIMER:

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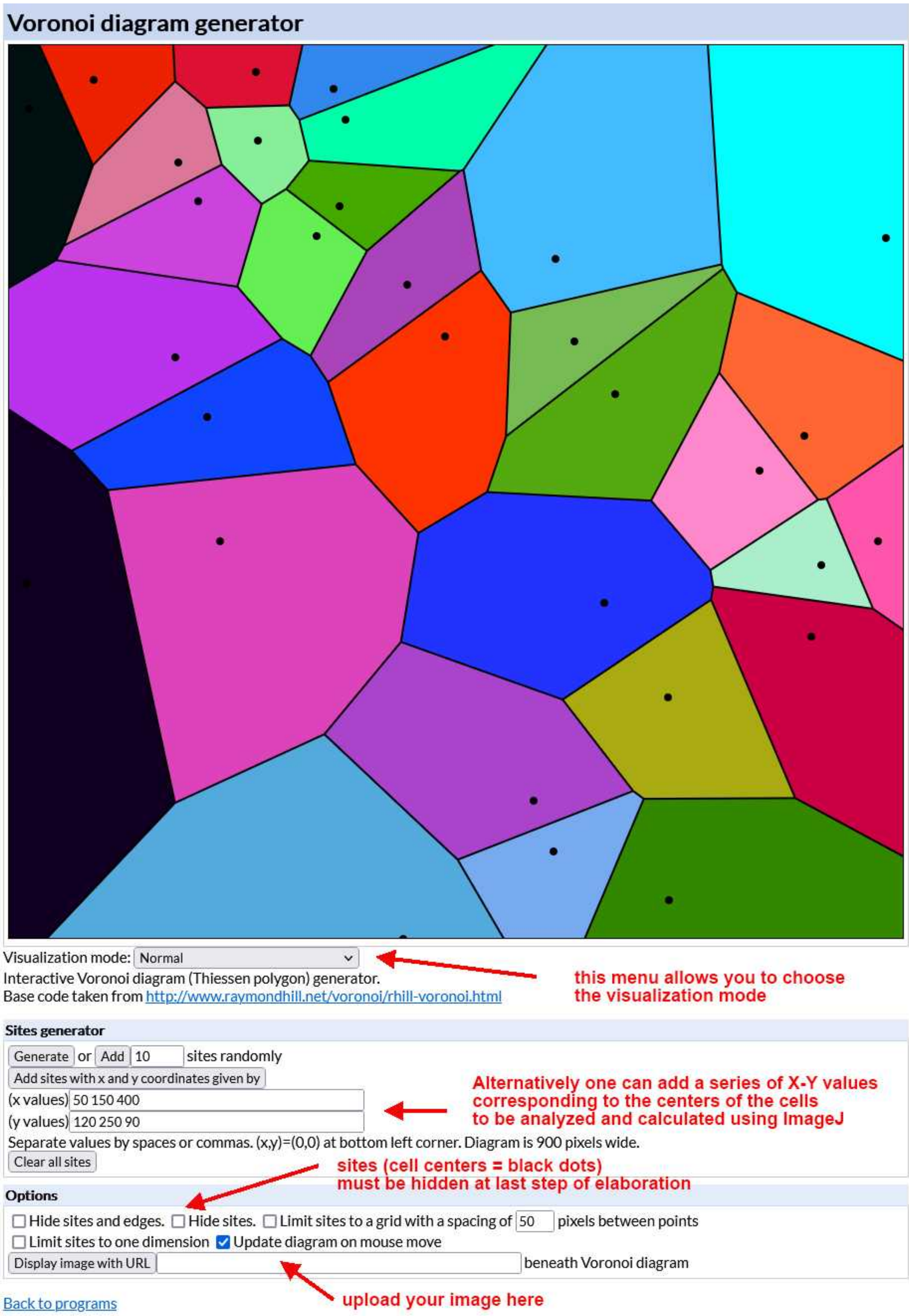
BEFORE STARTING

Be sure to familiarize yourself with the theory of Voronoi tessellation

Image processing with the **Voronoi generator**

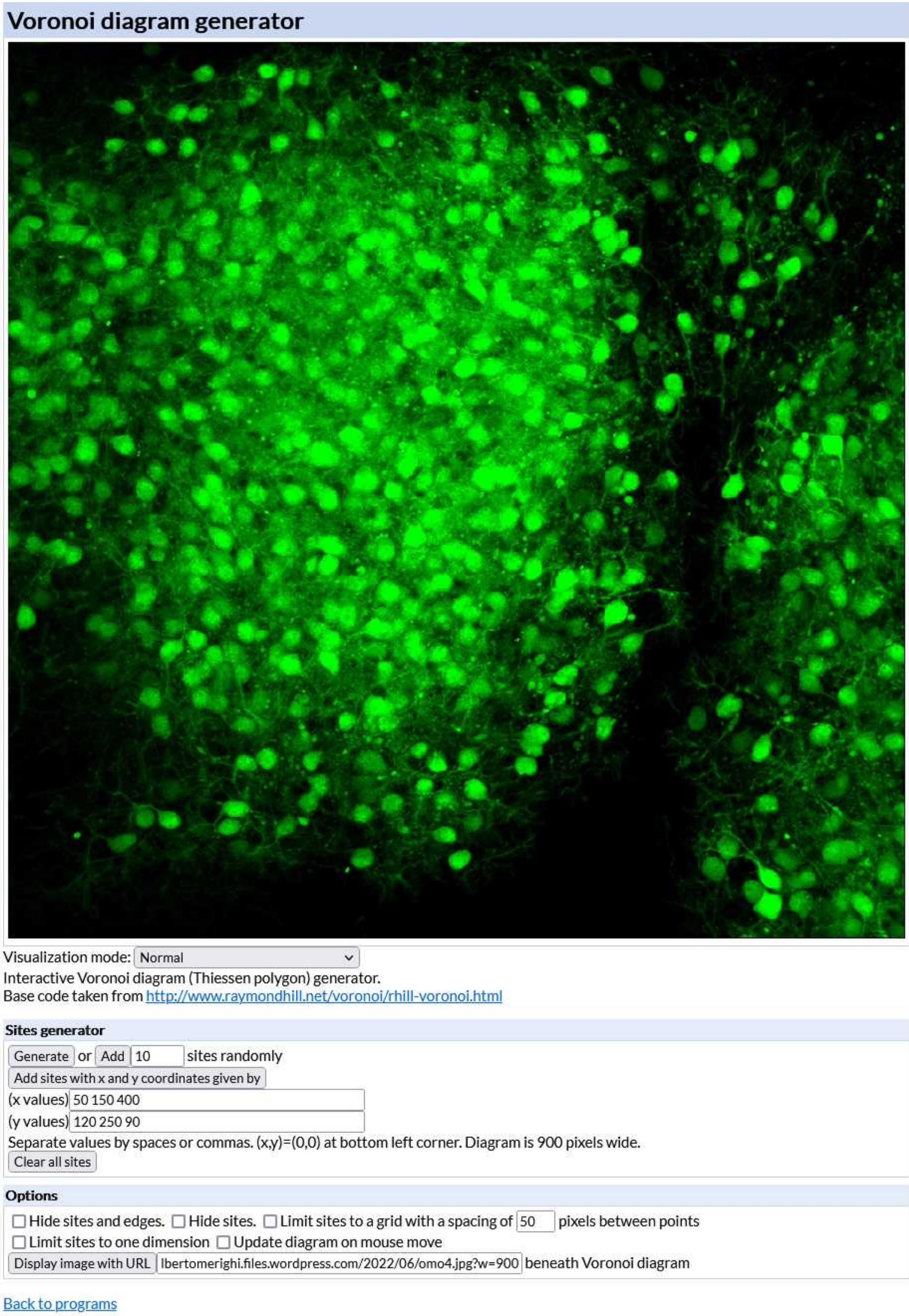
1 Open the interactive Voronoi diagram (Thiessen polygon) generator (https://cfbrasz.github.io/Voronoi.html).



The aspect of the Voronoi diagram generator mask

2 Upload the image to be analyzed as indicated in the figure above. To do so your image (size must be 900x900 pixels and preferably saved as a PNG file) has to be uploaded to the internet first so that it is possible to copy and paste its URL in the Voronoi generator. After uploading the

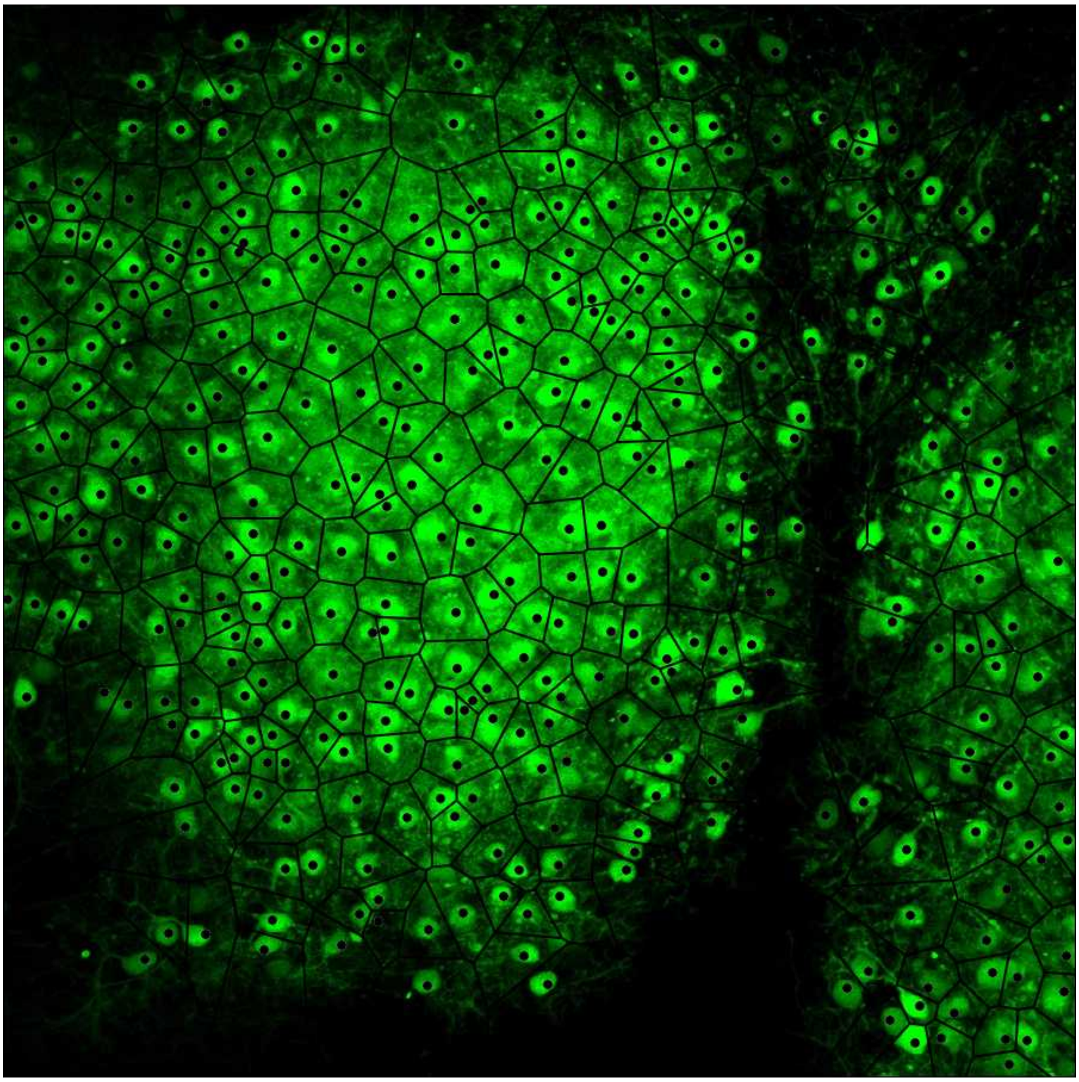
generator displays the image in its working space as shown in the figure below.



The image to be analyzed is uploaded to the Voronoi generator

3 Using the mouse click above the center of each cell to generate the Voronoi polygons. In the end,

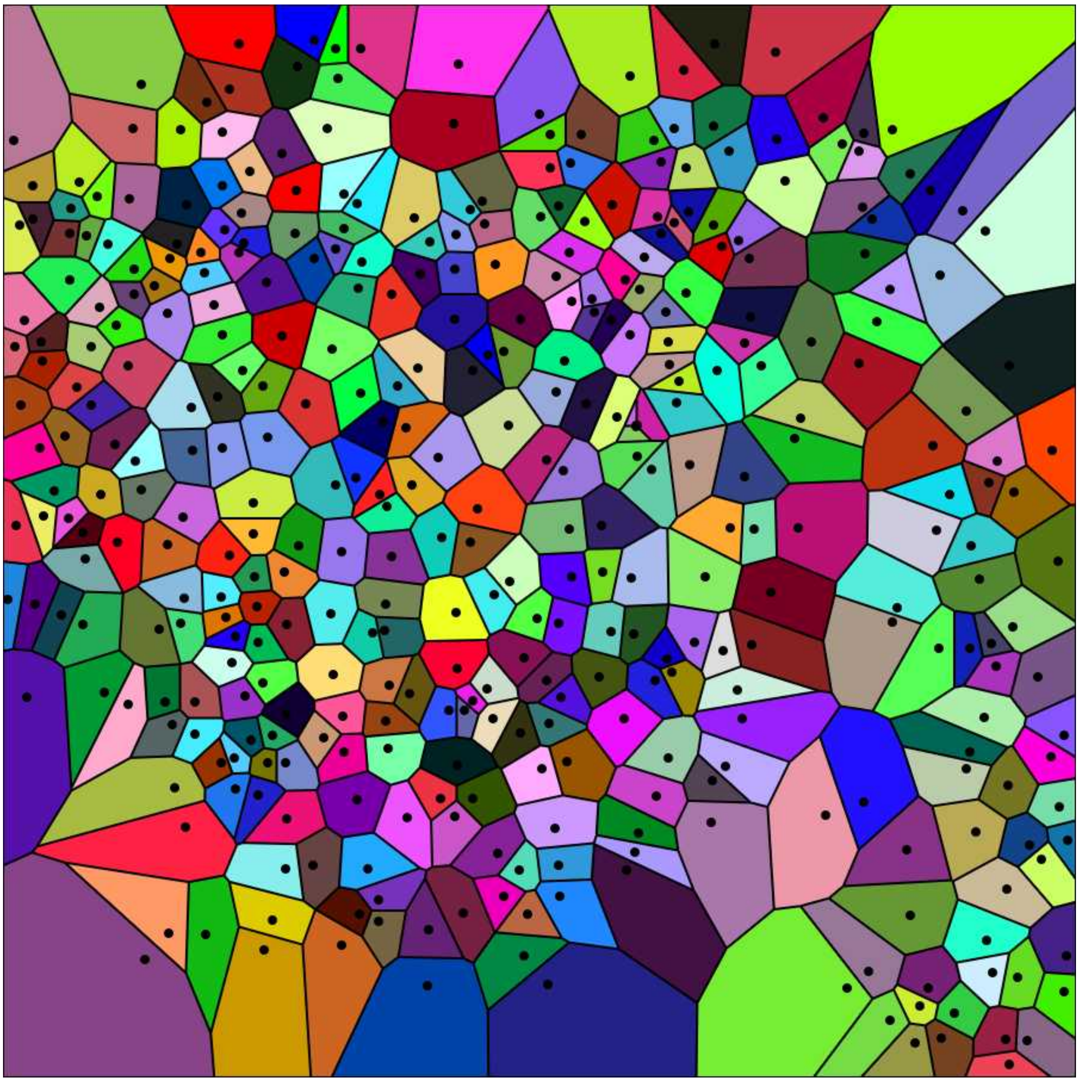
you will obtain the image shown below. Save the image on your computer (right-click on the image and choose "save" from the drop-down menu).



Voronoi tesselation of the uploaded image

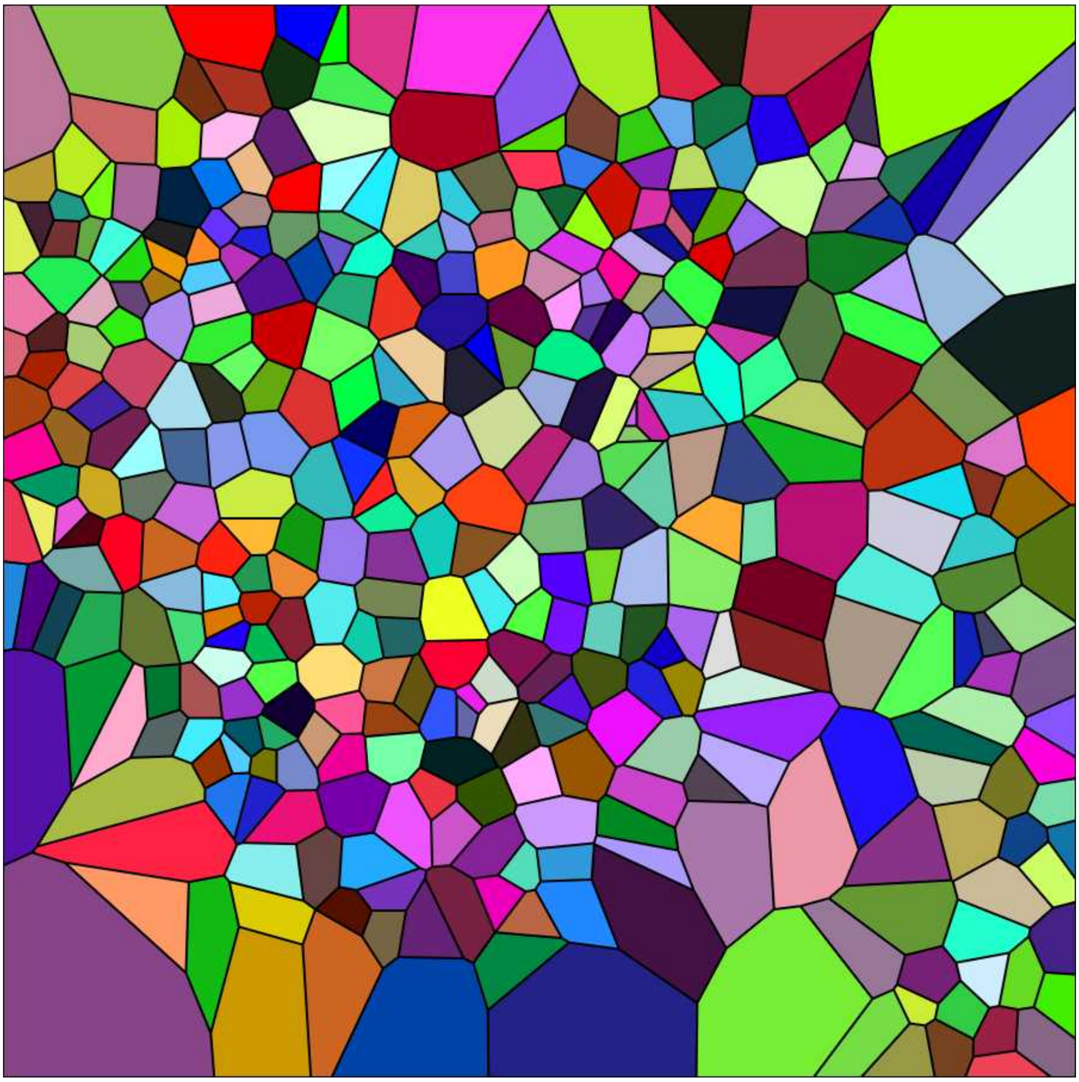
4 Choose "Visualization Normal" from the Visualization mode drop-down menu of the generator. The tesselation appears as shown in the image below. Again save the image on your computer (right-click on the image and choose "save" from the drop-down menu).

Citation: adalberto.merighi, Laura Lossi Voronoi tesselation https://dx.doi.org/10.17504/protocols.io.yxmvmnrx6g3p/v1



Voronoi polygons in the Normal visualization mode (Elaboration 1)

5 Select "Hide sites" from the Options menu of the generator. The tesselation appears as shown in the image below (black dots corresponding to cell centers disappear). Again save the image on your computer (right-click on the image and choose "save" from the drop-down menu).



Voronoi tesselation with cell sites hidden (Elaboration 2)

Elimination of the marginal polygons with **Photoshop**

6

Due to the properties of the Voronoi partition, some polygons of the paving are not statistically representative of the set of polygons - see Marcelpoil, R.; Usson, Y. (1992) Methods for the study of cellular sociology: Voronoi diagrams and parametrization of the spatial relationships. *Journal of Theoretical Biology* **154**, 359-369.
Those polygons are associated with points located on the border of the cell population and have one or more summits that do not contain total information on their "surround" (***marginal polygons***). Such summits are created by points that belong to a half-plane that does not contain this particular summit. Therefore, every point of the cell population whose associated polygon satisfies one of the two following conditions is not taken into account in the further calculations.
- The polygon is open (the point belongs to the convex hull),
- At least one of the summits of the polygon is outside the convex hull.

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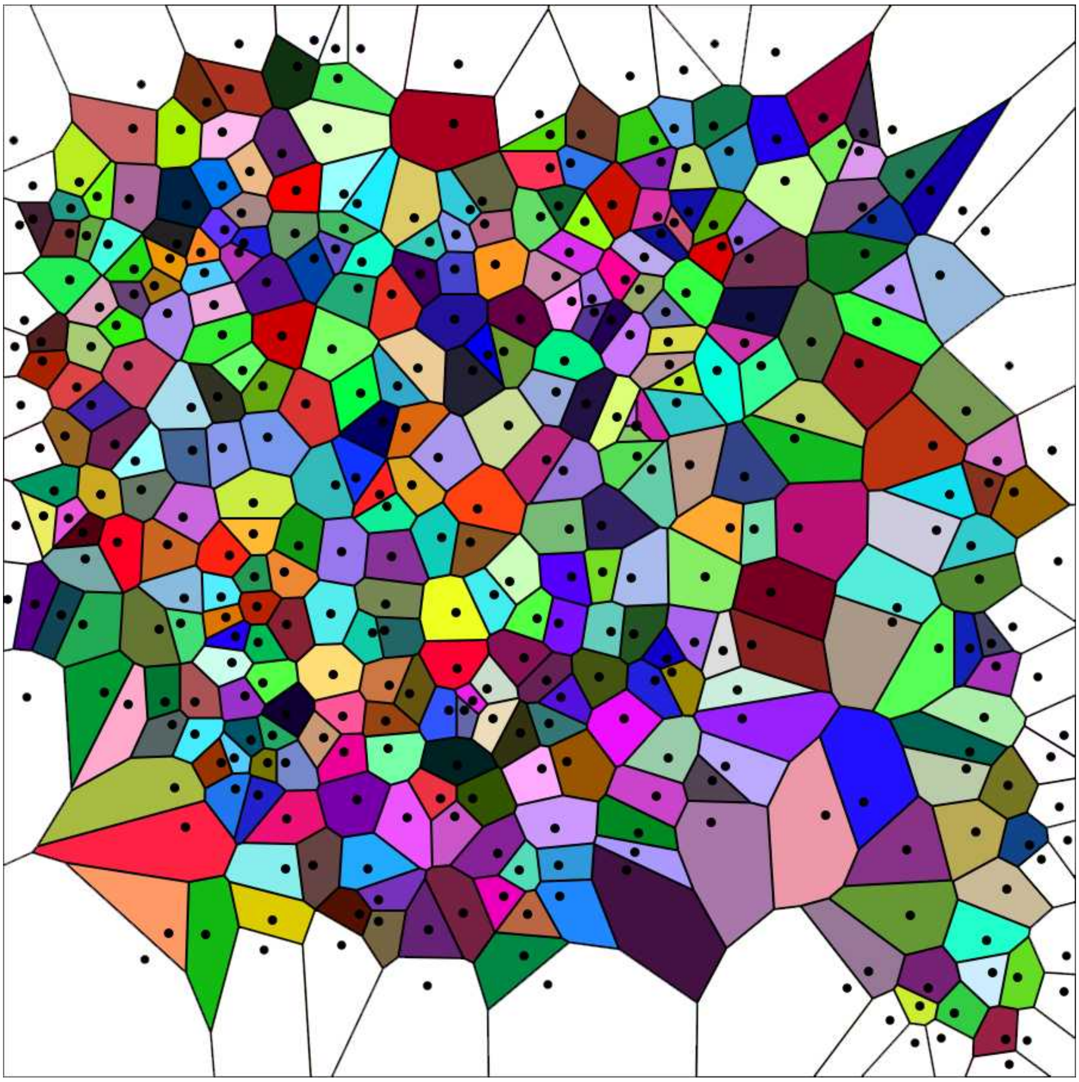
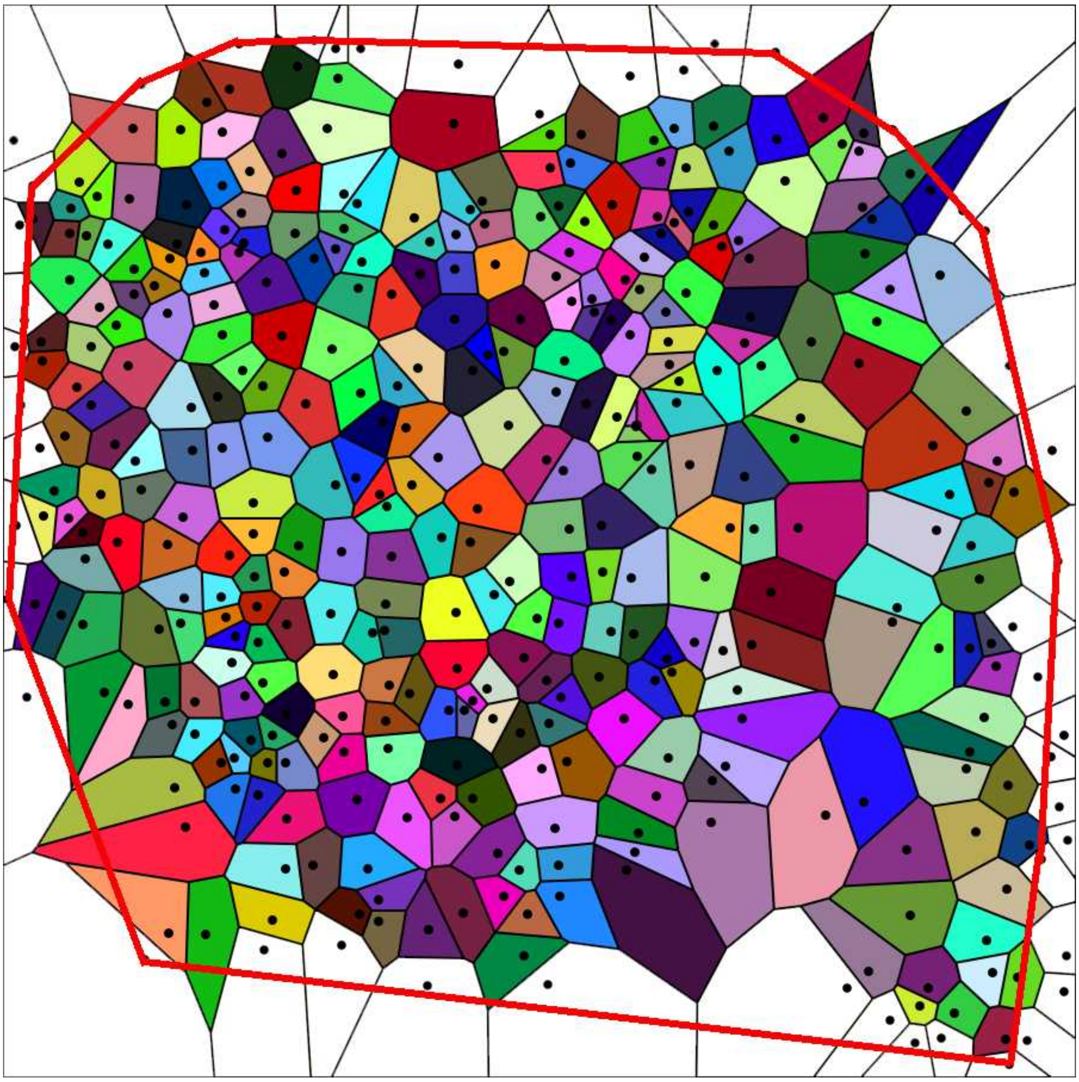
6.1 Elimination of the open polygons is carried out with Photoshop using the Magic wand tool to select and cancel them from the image above named Elaboration 1, as shown in the image below.



Elimination of the polygons with an open side, i.e. a side that is part of the border of the image (Elaboration 3)

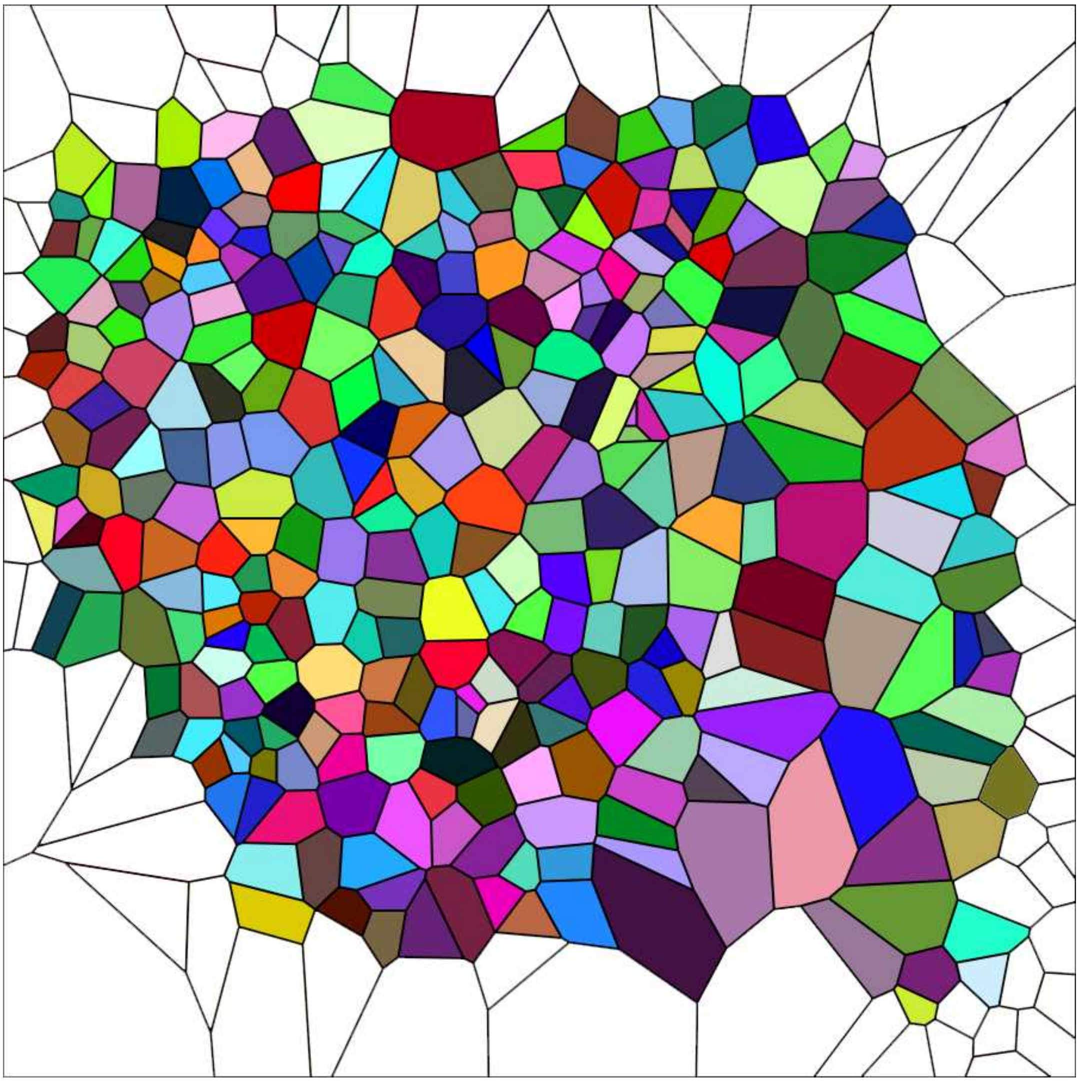
7 Construct the ***convex hull*** from the image Elaboration 3. The convex hull is constructed with the Line tool by drawing segments that join the site points (cell centers) of the eliminated open polygons so that there are no concavities, as shown in the image below.

Citation: adalberto.merighi, Laura Lossi Voronoi tesselation https://dx.doi.org/10.17504/protocols.io.yxmvmnrx6g3p/v1



Construction of the convex hull

8 Eliminate the polygons intersected by the convex hull and the polygons with open sides using the image Elaboration 2 (without cell sites) as shown in the image below.



All marginal polygons are eliminated (Elaboration 4)

9 Cancel the sides of the marginal polygons. Use the Magic wand tool of photoshop followed by the commands Selection → **Expand** 2px; Selection → **Contract** 1px; **Cancel**; Modify → **Stroke (color black)** 2px. You should obtain an image in which the area of the marginal polygons is empty as in the figure below. This is the last elaboration that will be used for the subsequent steps of analysis.

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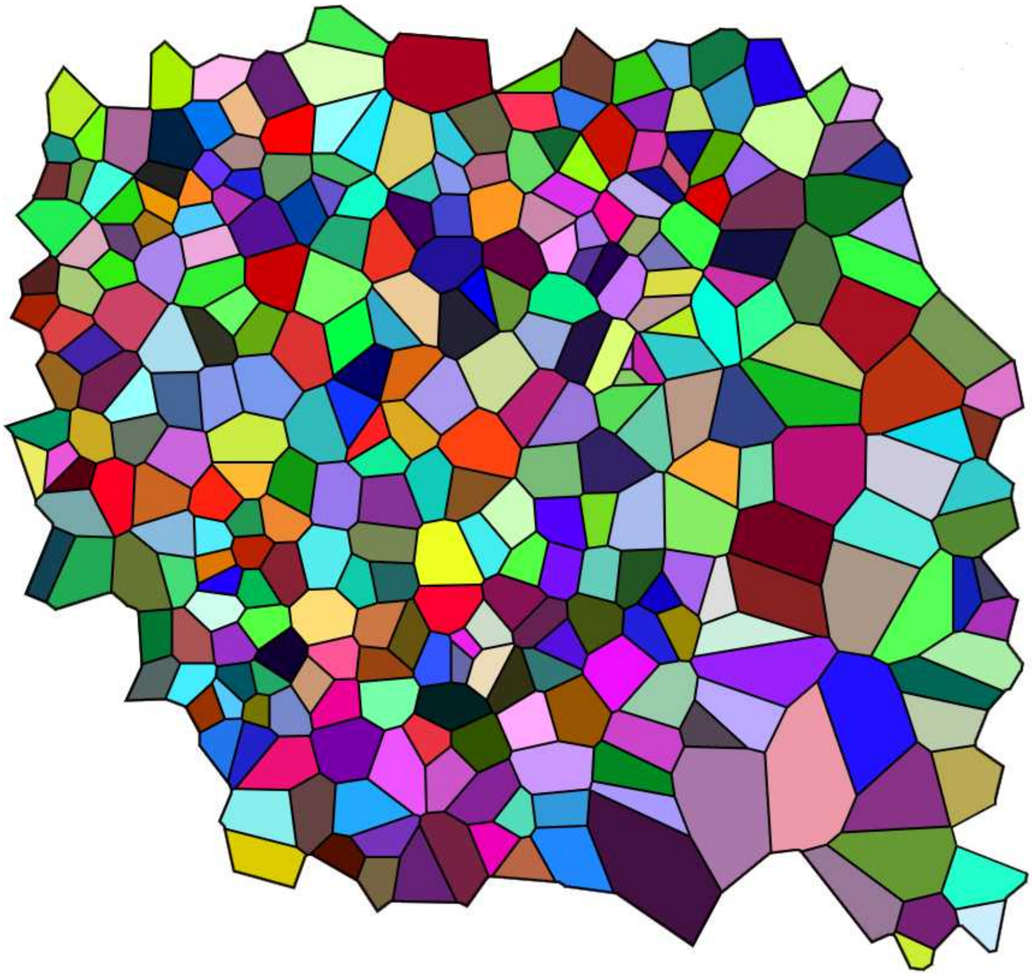

Last elaboration of Voroni tessalation (Elaboration 5)

Analysis of Voronoi polygons with **ImageJ**

10 **Open** the image to be analyzed with ImageJ. Set the appropriate scale with **Analyze → Set scale**.
Run the following Macro by selecting **Plugins → Macros → Run → Voronoi Macro**

Voronoi Macro

```
run("Enhance Contrast...", "saturated=2");
run("8-bit");
run("Find Edges");
//run("Brightness/Contrast...");
setMinAndMax(0, 0);
run("Apply LUT");
run("Set Measurements...", "area perimeter shape limit display redirect=None decimal=6");
run("Analyze Particles...", "display summarize add in_situ");
```

The macro enhances image contrast (optional), converts the image into a B&W 8-bit image, finds

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the edges of the Voronoi polygons, and optimizes their contrast as in the figure below.

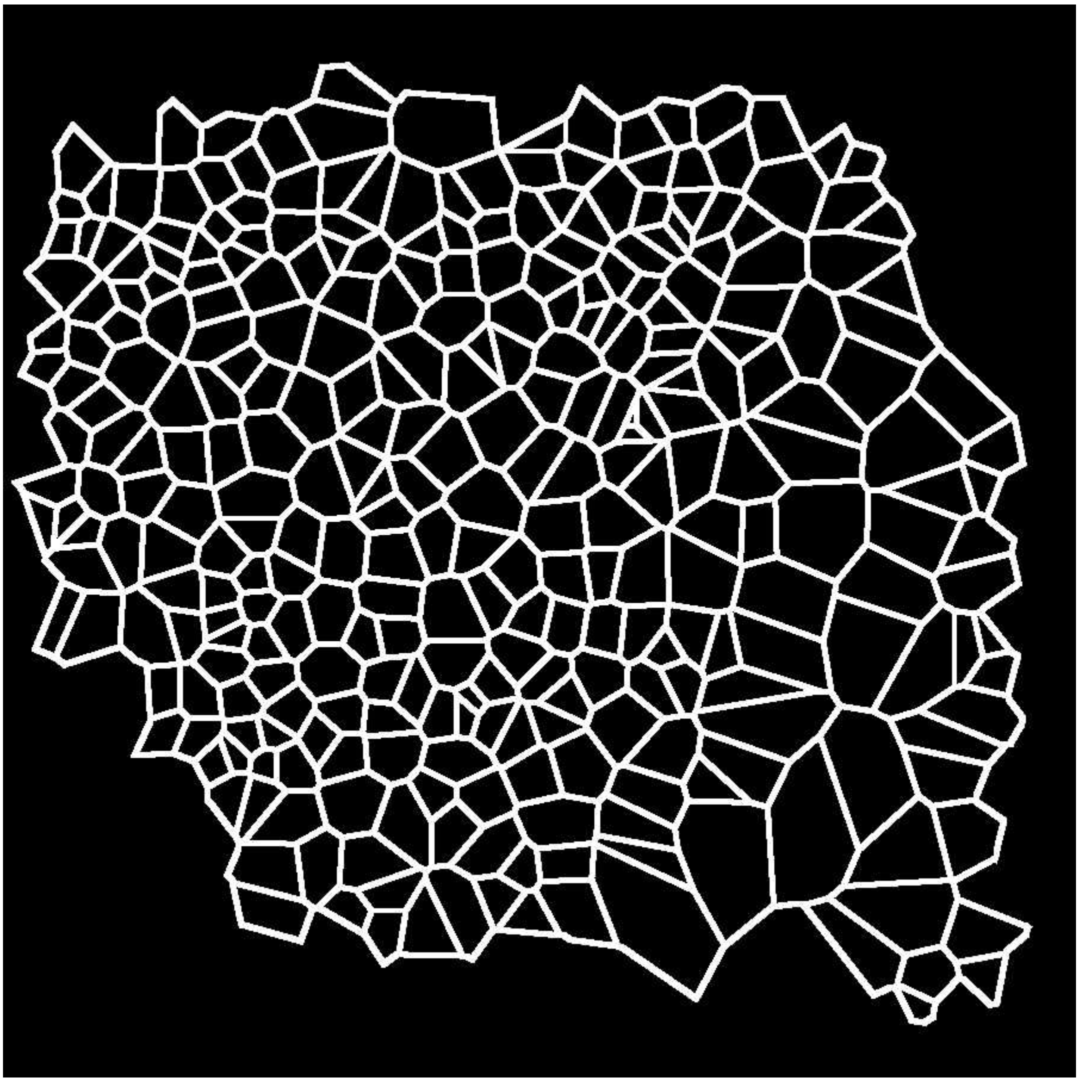

Image of Voronoi polygons after application of the **Find Edges** command and optimization of contrast (Elaboration 6)

It then sets up the measurements necessary for the following analysis of polygons: **Area**, **Shape descriptors**, and **Perimeter**. It also permits the creation of an image (below) with the overlay indication of the individual polygons that the program has measured (**Add to overlay** and **Display label**). It finally sets the number of **Decimal places** to 6. When run, the Macro performs the command **Analyze Particles**.

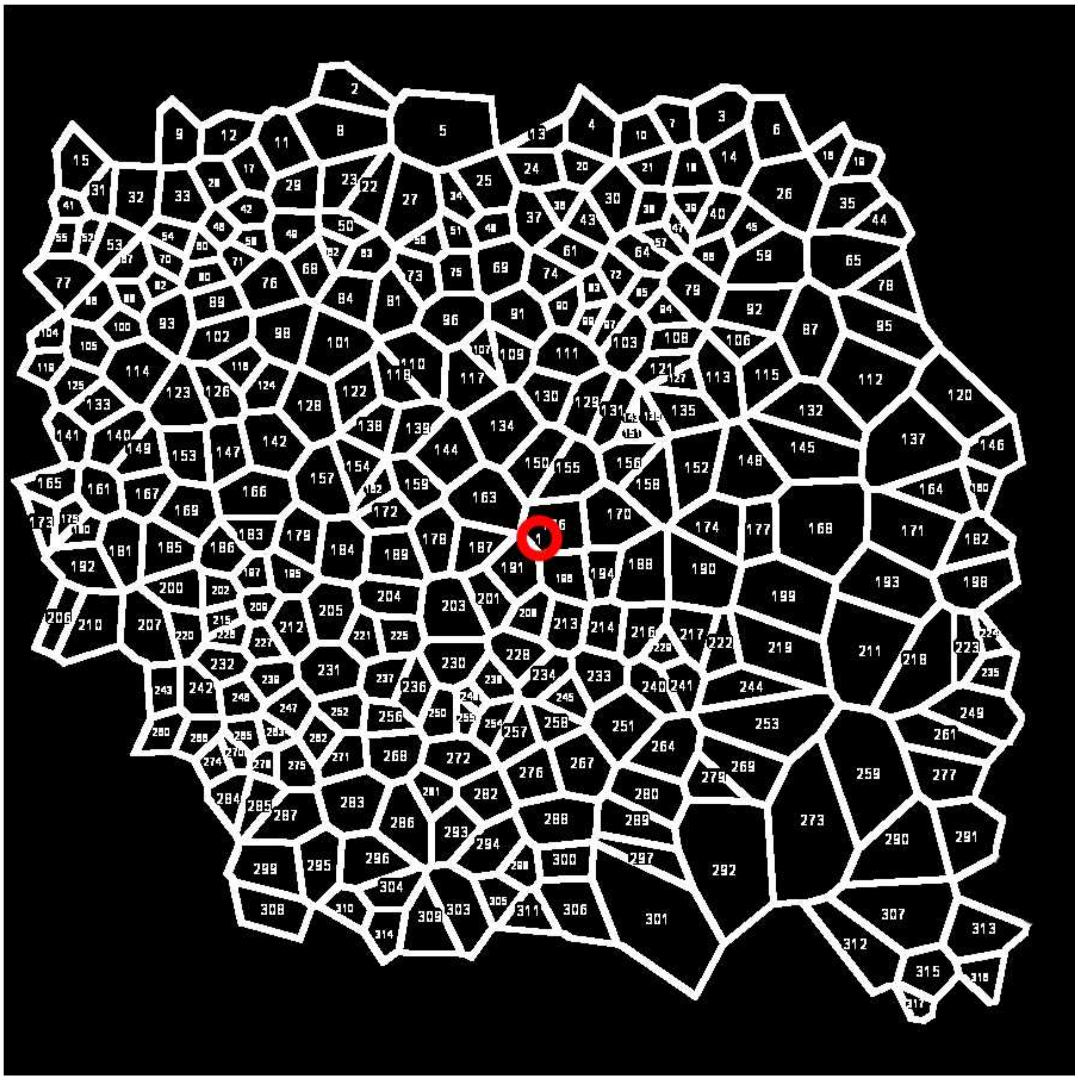

Image of the Voronoi polygons after performing the **Analyze Particles** command. Each polygon is assigned a progressive number. Note the number 1 at the center of the image (encircled in red). This corresponds to the first counted particle that the program considers being the ensemble of the marginal polygons (highlighted in red in the following image). ***Note that the red circle is added here for clarity but the program does not display it at all.***

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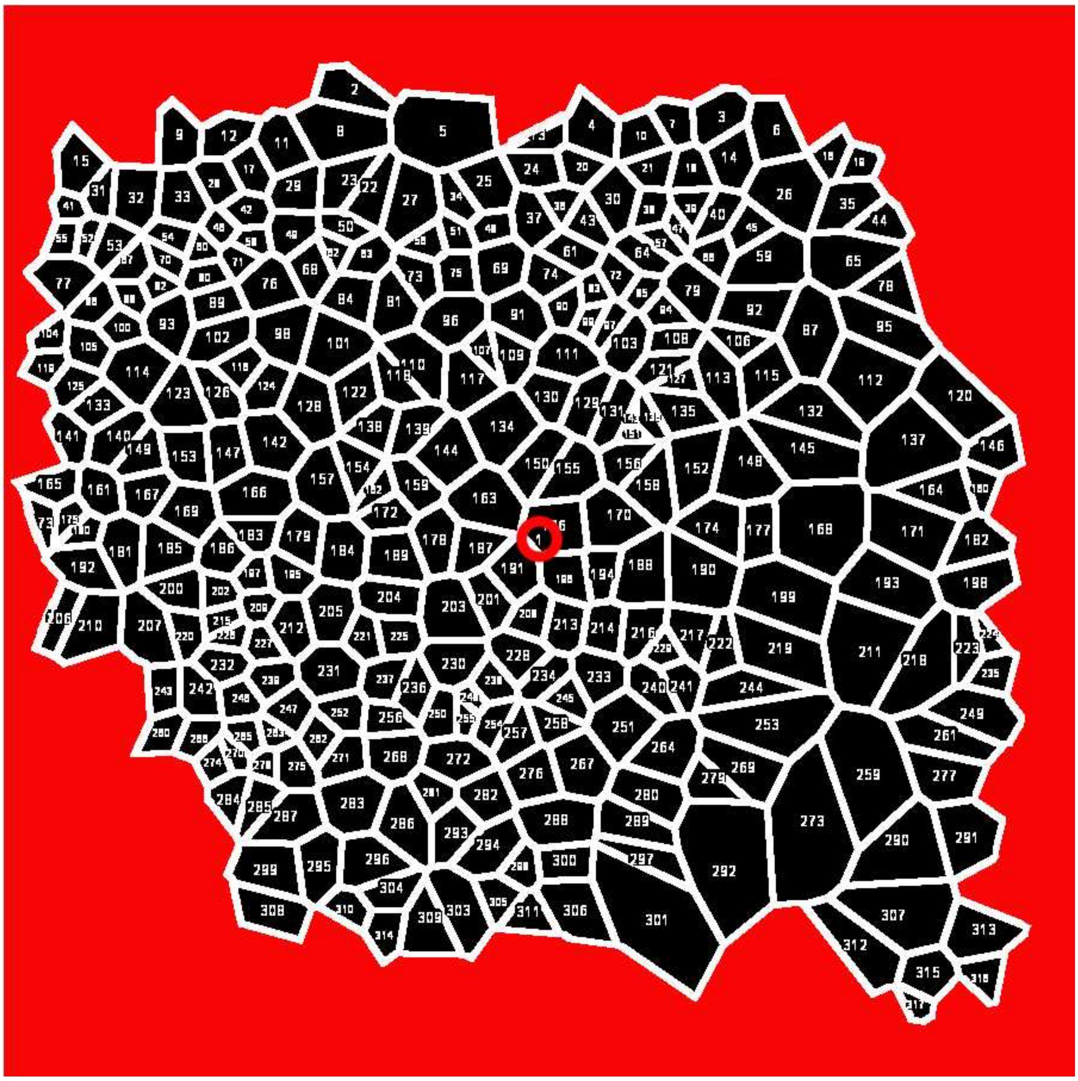

The image displays in red the particle that the program numbers as particle 1 that must be discarded in the following analysis.

At the end of the Macro, all computed values are saved in a .csv or a .xls file (according to the version of ImageJ used) that must be converted into a .xlsx Excel file.

Analysis of data

11 Open the .csv or .xls file generated by ImageJ with Microsoft excel. The file appears as follows

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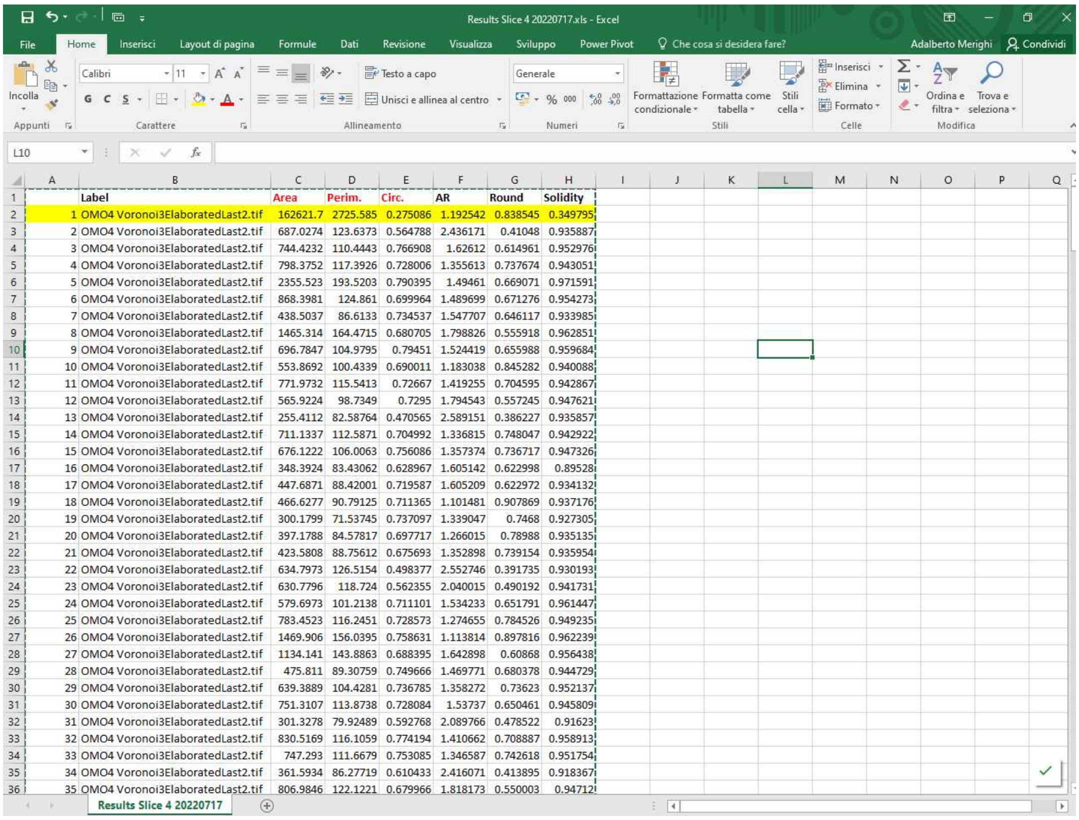

A	Label	Area	Perim.	Circ.	AR	Round	Solidity
1	OMO4 Voronoi3ElaboratedLast2.tif	162621.7	2725.585	0.275086	1.192542	0.838545	0.349795
2	OMO4 Voronoi3ElaboratedLast2.tif	687.0274	123.6373	0.564788	2.436171	0.41048	0.935887
3	OMO4 Voronoi3ElaboratedLast2.tif	744.4232	110.4443	0.766908	1.62612	0.614961	0.952976
4	OMO4 Voronoi3ElaboratedLast2.tif	798.3752	117.3926	0.728006	1.355613	0.737674	0.943051
5	OMO4 Voronoi3ElaboratedLast2.tif	2355.523	193.5203	0.790395	1.49461	0.669071	0.971591
6	OMO4 Voronoi3ElaboratedLast2.tif	868.3981	124.861	0.699964	1.489699	0.671276	0.954273
7	OMO4 Voronoi3ElaboratedLast2.tif	438.5037	86.6133	0.734537	1.547707	0.646117	0.933985
8	OMO4 Voronoi3ElaboratedLast2.tif	1465.314	164.4715	0.680705	1.798826	0.555918	0.962851
9	OMO4 Voronoi3ElaboratedLast2.tif	696.7847	104.9795	0.79451	1.524419	0.655988	0.959684
10	OMO4 Voronoi3ElaboratedLast2.tif	553.8692	100.4339	0.690011	1.183038	0.845282	0.940088
11	OMO4 Voronoi3ElaboratedLast2.tif	771.9732	115.5413	0.72667	1.419255	0.704595	0.942867
12	OMO4 Voronoi3ElaboratedLast2.tif	565.9224	98.7349	0.7295	1.794543	0.557245	0.947621
13	OMO4 Voronoi3ElaboratedLast2.tif	255.4112	82.58764	0.470565	2.589151	0.386227	0.935857
14	OMO4 Voronoi3ElaboratedLast2.tif	711.1337	112.5871	0.704992	1.336815	0.748047	0.942922
15	OMO4 Voronoi3ElaboratedLast2.tif	676.1222	106.0063	0.756086	1.357374	0.736717	0.947326
16	OMO4 Voronoi3ElaboratedLast2.tif	348.3924	83.43062	0.628967	1.605142	0.622998	0.89528
17	OMO4 Voronoi3ElaboratedLast2.tif	447.6871	88.42001	0.719587	1.605209	0.622972	0.934132
18	OMO4 Voronoi3ElaboratedLast2.tif	466.6277	90.79125	0.711365	1.101481	0.907869	0.937176
19	OMO4 Voronoi3ElaboratedLast2.tif	300.1799	71.53745	0.737097	1.339047	0.7468	0.927305
20	OMO4 Voronoi3ElaboratedLast2.tif	397.1788	84.57817	0.697717	1.266015	0.78988	0.935135
21	OMO4 Voronoi3ElaboratedLast2.tif	423.5808	88.75612	0.675693	1.352898	0.739154	0.935954
22	OMO4 Voronoi3ElaboratedLast2.tif	634.7973	126.5154	0.498377	2.552746	0.391735	0.930193
23	OMO4 Voronoi3ElaboratedLast2.tif	630.7796	118.724	0.562355	2.040015	0.490192	0.941731
24	OMO4 Voronoi3ElaboratedLast2.tif	579.6973	101.2138	0.711101	1.534233	0.651791	0.961447
25	OMO4 Voronoi3ElaboratedLast2.tif	783.4523	116.2451	0.728573	1.274655	0.784526	0.949235
26	OMO4 Voronoi3ElaboratedLast2.tif	1469.906	156.0395	0.758631	1.113814	0.897816	0.962239
27	OMO4 Voronoi3ElaboratedLast2.tif	1134.141	143.8863	0.688395	1.642898	0.60868	0.956438
28	OMO4 Voronoi3ElaboratedLast2.tif	475.811	89.30759	0.749666	1.469771	0.680378	0.944729
29	OMO4 Voronoi3ElaboratedLast2.tif	639.3889	104.4281	0.736785	1.358272	0.73623	0.952137
30	OMO4 Voronoi3ElaboratedLast2.tif	751.3107	113.8738	0.728084	1.53737	0.650461	0.945809
31	OMO4 Voronoi3ElaboratedLast2.tif	301.3278	79.92489	0.592768	2.089766	0.478522	0.91623
32	OMO4 Voronoi3ElaboratedLast2.tif	830.5169	116.1059	0.774194	1.410662	0.708887	0.958913
33	OMO4 Voronoi3ElaboratedLast2.tif	747.293	111.6679	0.753085	1.346587	0.742618	0.951754
34	OMO4 Voronoi3ElaboratedLast2.tif	361.5934	86.27719	0.610433	2.416071	0.413895	0.918367
35	OMO4 Voronoi3ElaboratedLast2.tif	806.9846	122.1221	0.679966	1.818173	0.550003	0.94712

Screenshot of the .xls file generated by ImageJ (the file is created from the analysis of the image elaborated as described above and contains 318 lines, i.e. the information about the 317 particles (polygons) counted by the program.

The file contains the following information: Column A: progressive numbering of the particles (polygons) counted by ImageJ; Column B: Identification of image analyzed; Column C: Area (in µm^2 if the **Set scale** command has been set properly); Column D: Perimeter (in µm if the **Set scale** command has been set properly); Column E: Circularity (or Roundness factor); Columns F-H: Other shape descriptors computed by ImageJ that are not used in the analysis. ***Note that line 2 (highlighted in yellow) corresponding to Particle 1 must be deleted*** (as indicated above).

Save the file as a .xlsx file.

12 Open the .xlsx file in Excel and calculate the following:

- ***Mean*** of **area**, **perimeter**, and **circularity** (**roundness**)
- ***Standard deviation*** of **area**, **perimeter**, and **circularity** (**roundness**)
- **Area Disorder (AD)**
- **Roundness Factor Homogeneity (RFH)**

The mean circularity (roundness) (**RFav**) is computed directly by the ImageJ program using the following formula

$$RFav = 1/N \sum(i = 1)^N 4\pi A(X_i)/(L(X_i)^2)$$

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where A(x) is the area and L(x) is the perimeter of the *N* polygons generated by the Voronoi generator. RF_{av} is a pure number ($0< RF_{av} \leq 1$).

The **AD** is calculated as follows:

$$AD = 1 - (1 + \sigma_A / A_a v)^-1$$

where σ_A is the area standard deviation, and A_{av} is the mean area.

The **RFH** is calculated as follows:

$$RFH = (1 + \sigma_R F / RF_a v)^-1$$

where σ_{RF} is the roundness factor standard deviation, and RF_{av} is the mean roundness factor. Both are pure numbers with values >0 and ≤1.

13 Transfer the values of RF_{av}, AD, and RFH to a new Excel spreadsheet for subsequent statistical analysis.

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