

Dot Analyzer for SEM micrographs (Version 3.1)

This document describes the workings of the ImageJ plugin "Dot Analyzer V3.1...", written by Philippe Girard (philippe.girard@ijm.fr)

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The plugin was written to analyse SEM micrograph as shown in Fig. 1.

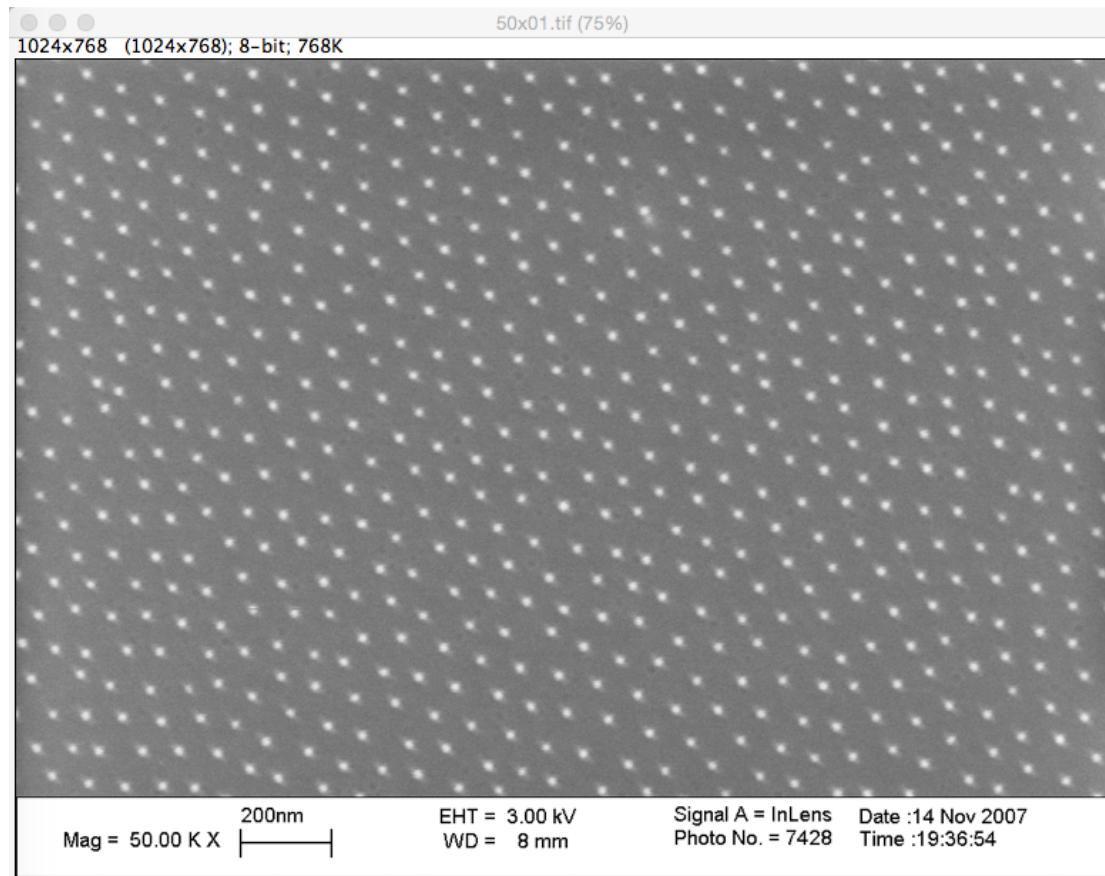


Fig. 1: A typical SEM micrograph.

1. Installation instructions

To install the plugin, just copy the file Dot_Analyzer.jar into the “plugins” directory, within the “ImageJ” directory. On PCs this directory is under “Program Files” and on a MacOSX it is under “Applications”. Both require certain privileges to do this.

Restart ImageJ. The plugin is installed under the menu:

“Plugins▷Dot Analyzer▷Dot Analyzer v3.1...”.

2. The “Parameters” main window.

When you start the plugin, you have to define different parameters for the analysis and to select the different diagrams/plots that you want to visualize (Fig. 2).

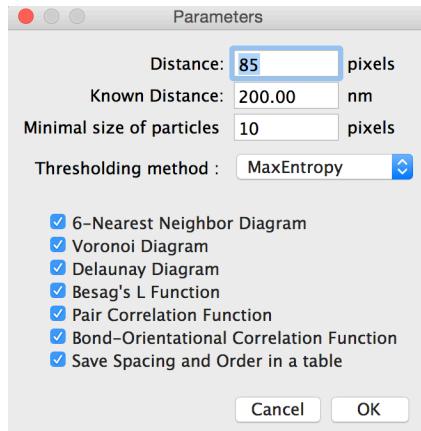
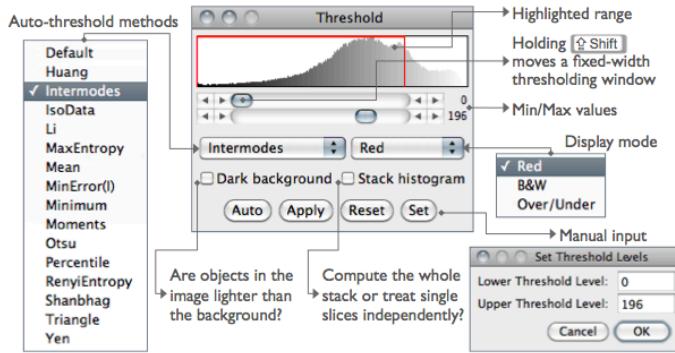


Fig. 2: The “Parameters” main window

You must indicate:

- 1) Distance in pixels and
- 2) Known Distance in nm that are used for conversion from pixel to nm. You should use the scale bar at the bottom left of the micrograph. For example, in the Heidelberg SEM data, the parameters are
 - 200 nm = 43 pixels at 25.00 KX magnification
 - 200 nm = 85 pixels at 50.00 K X magnification
 - 200 nm = 171 pixels at 100.00 KX magnification
 - 100 nm = 171 pixels at 200.00 KX magnification
- 3) Set the minimum size (in pixels^{^2}) to exclude objects that appear in the binary image that are clearly not objects of interest (**Attention:** this parameter is clearly connected to the thresholding method you have selected). This parameter is the same that appears in “Size (^2)” (where you have to define the minimum and the maximum size) of the “Analyze Particles” window.
- 4) Select the Thresholding method: the list is the same that the one you can find directly in the Threshold window (Image>Adjust>**Threshold...**) as indicated below. The “MaxEntropy” method gives the best result on the Fig 1 but you have to consider trying different auto-threshold method. Here for example, the methods: “Default”, “IJ_IsoData” (=“Default”), “MaxEntropy”, “RenyiEntropy” and “Yen” are also good. If the threshold method you have selected do not give you the best result, you do not need to stop the plugin because it will ask you if you want to restart the analysis after the first step.



- 5) Select “6-nearest neighbour diagram” if you want to visualize the 6 closest neighbours of each spots (see Fig. 3). In the analysis, the spots which are too closed to the borders are not taken into account because they do not have 6-closest neighbours as you can see on the Fig 3. Each spots is connected to its 6 neighbours with a line of different colours (red, orange, yellow, green, magenta, blue).

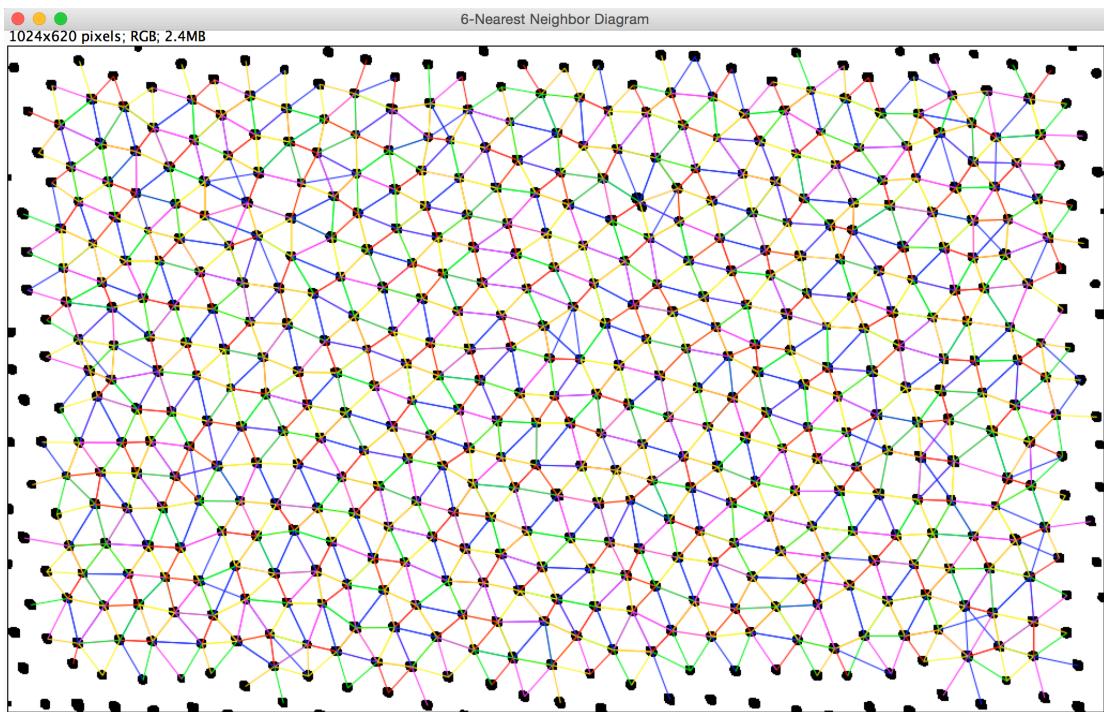


Fig. 3: 6-nearest neighbor diagram of the Fig. 1

- 6) “Voronoi diagram (or tessellation)” [1-4] (Fig. 4 and Fig. 5). The Voronoi diagram is a simple mathematical construct that has proved useful in fields as diverse as environmental studies, cell biology, crystallography, transportation planning, and communications theory. Given a set of points (the center of mass of each gold-dot), the Voronoi diagram defines a series of cells surrounding each point. Each cell contains all points that are closer to its defining point than to any other point in the set. Subsequently, the “borders” of the cells are equidistant between the defining points of adjacent cells.

By doing so, the number of borders give you the number of closest neighbors. The difference by the previous diagram (6 closest-neighbor diagram) is that you do not force each spot to have 6 neighbors. For patterns that contain non-uniform defect structures as disinclination, this diagram is more appropriated than the previous one.

For more information about the Voronoi diagram, see the Wikipedia webpage http://en.wikipedia.org/wiki/Voronoi_diagram

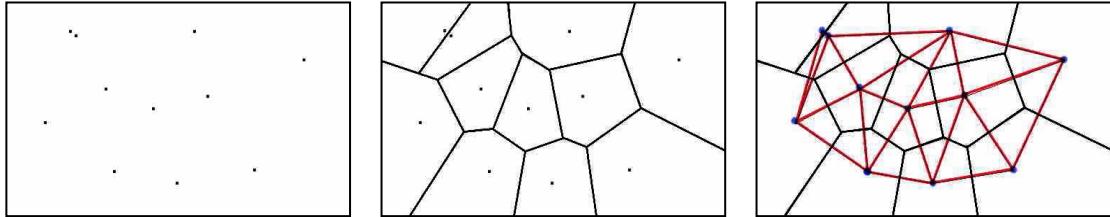


Fig. 4: (Left) Points that make the Voronoi diagram. (Center) A Voronoi diagram constructed for those points. (Right) Delaunay in red and Voronoi in black constructed together on the same graphic.

- 7) "Delaunay diagram (or triangulation)" [3-4] (Fig. 4 and Fig. 5). In graph theory, the Delaunay triangulation corresponds to the dual graph of the Voronoi tessellation. By dual, I mean to draw a line segment between two Voronoi vertices if their Voronoi polygons have a common edge, or in more mathematical terminology: there is a natural bijection between the two which reverses the face inclusions. This diagram gives the distance between the closest neighbors of each gold-dot.
- http://en.wikipedia.org/wiki/Delaunay_triangulation

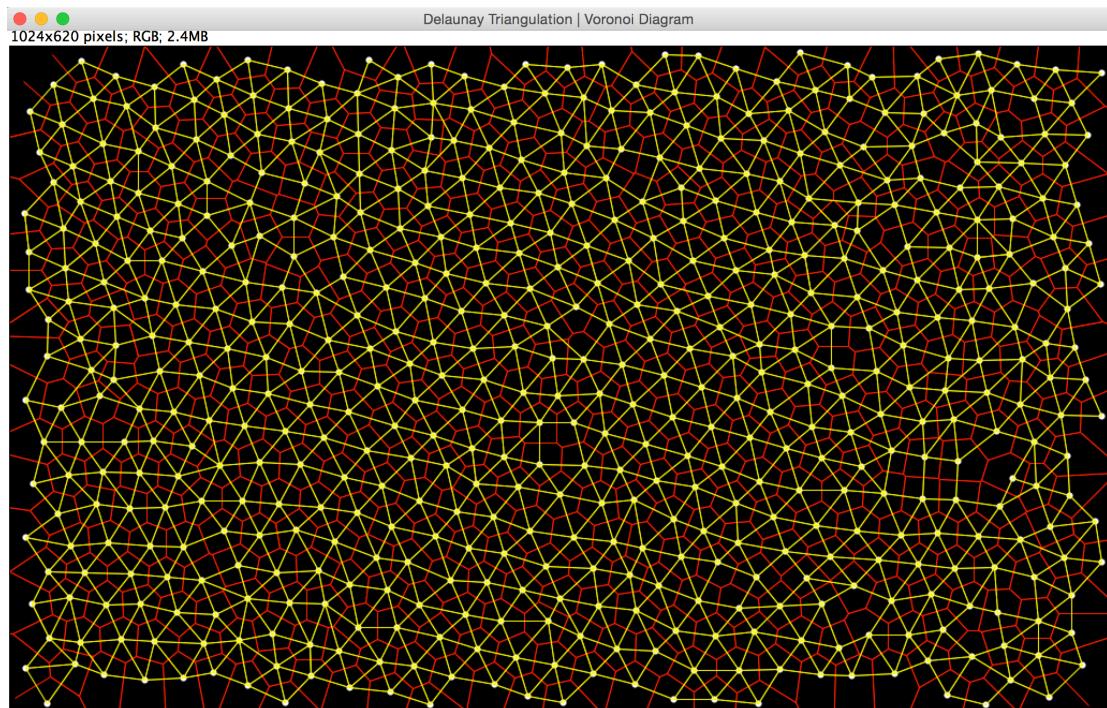


Fig. 5: Delaunay triangulation (in yellow) and Voronoi tessellation (in red) of points (in white) that define the dot positions of the Fig. 1. You can choose to represent only the Voronoi tessellation or the Delaunay triangulation.

- 8) "Besag's L Function" (Fig. 6): The Besag's L Function is based on the Ripley's K Function. Details of various theoretical aspects of K are in books [5-7]. Ripley's K function is a popular tool to analyze mapped spatial point pattern. It is defined without edge correction as:

$$K(r) = \int_{\rho=0}^r g(\rho) 2\pi \rho d\rho$$

If points are distributed independently from each other, $g(\rho)=1$ for all values of ρ , so $K(r) = \pi r^2$

This value is used as a benchmark:

- $K(r) > \pi r^2$ indicates that the average value of $g(\rho)$ is greater than 1. The probability to find a neighbor at the distance ρ is then greater than the probability to find a point in the same area anywhere in the domain: points are aggregated.

- Inversely $K(r) < \pi r^2$ indicates that the average neighbor density is smaller than the average point density on the studied domain. Points are dispersed.

$K(r)$ is estimated by the ratio of the average number of neighbors on the density, estimated itself by the total number of points divided by the domain area λ which is the density (number per unit area = $N/\text{area of the picture}$) of event. Given that only the points in a bounded window of observation can be studied, edge correction is necessary to obtain precise estimates. The weighted edge-corrected function $K(r)$ is defined as:

$$K(r) = \frac{1}{\lambda} \sum_{i=1}^N \sum_{\substack{j=1 \\ i \neq j}}^N \frac{I(r_{ij} \leq r)}{w_{ij}(r) N}$$

where $I(r_{ij} \leq r)$ is an indication function with values either 0 if the condition does not hold or 1 if the condition holds and where the weight $w_{ij}(r)$ is the proportion of the circumference of a circle centered at the gold-dot i passing through the gold-dot j and that is inside the region of interest (ROI), which is defined as

$$w_{ij}(r) = \begin{cases} 1, & \text{if } r_{ij} \leq r_{ib} \\ 1 - \cos^{-1} \left(\frac{r_{ib}}{r} \right) \frac{1}{\pi}, & \text{if } r^2 \leq r_{ib1}^2 + r_{ib2}^2 \\ 1 - [\cos^{-1} \left(\frac{r_{ib1}}{r} \right) + \cos^{-1} \left(\frac{r_{ib2}}{r} \right) + \frac{\pi}{2}] \frac{1}{2\pi}, & \text{if } r^2 > r_{ib1}^2 + r_{ib2}^2 \end{cases}$$

where r_{ib} is the distance from the gold-dot i to the nearest boundary, r_{ib1} and r_{ib2} are the distances from gold-dot i to the nearest two boundaries. The first case when the circle is within the ROI, the second case is when the circle intersects with only one border and the last case is when the circle intersects two borders in a corner. Note that $w_{ij}(r)$ could be unbounded as r increases in practice. Following the recommendation by Ripley, $w_{ij}(r)$ could be restricted to be less than or equal to 4 for the gold-dot i having distance to j greater than the distance from the gold-dot i to the nearest boundary.

The Besag's L function is just a normalization of the Ripley's function:

$$L(r) = \sqrt{\frac{K(r)}{\pi}} - r$$

because for a homogenous Poisson process, the Ripley function is $K(r) = \pi r^2$ So the Besag's function is a measure of the deviation from a Poisson distribution and it is very useful because $L(r)$ has the advantage of linearizing

$K(r)$ and stabilizing its variance and has an expected value of zero for Poisson distribution.

So $L(r)$ can also be considered as a measure of the clusterization.

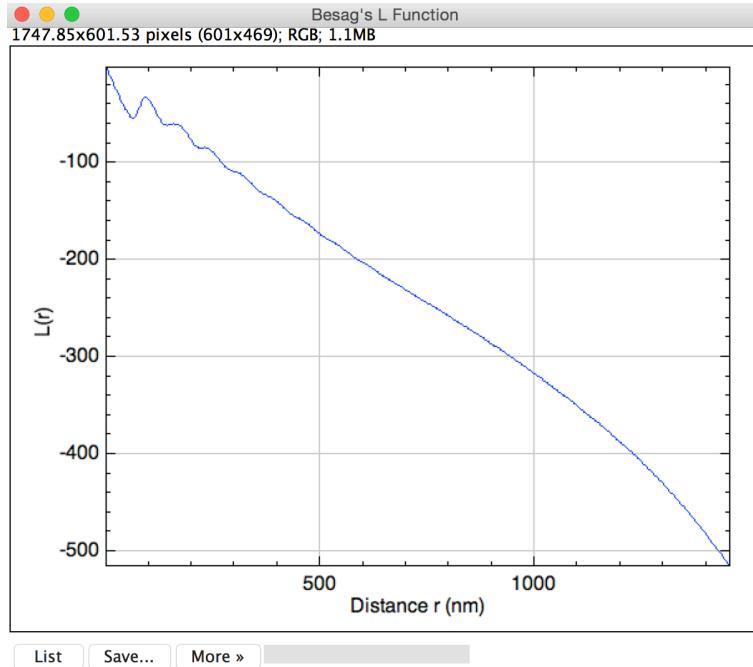


Fig. 6: Besag's L Function $L(r)$ of Fig. 1

9) "Pair correlation function" (Fig. 7) is measured with the Epanechnikov kernel [8] and an Ohser-Stoyan edge corrector factor [9].

The estimation of the pair correlation function $g(r)$ can be obtained by determining all pairs of gold-dots having inter-gold-dot distance in some small interval and counting their numbers. Since $g(r)$ is a density function, a more elegant method can be employed. Following the recommendation of Penttinen et al. [6], a kernel estimator is used for $g(r)$. The chosen kernel function is the Epanechnikov kernel:

$$w(r) = \begin{cases} \frac{3}{4\delta} \left(1 - \frac{r^2}{\delta^2}\right), & \text{if } |r| < \delta \\ 0, & \text{otherwise} \end{cases}$$

The kernel δ is very important because it determines the degree of smoothness of the function. Based on Penttinen et al. [8], set $\delta = 0.15/\sqrt{\lambda}$ in this study. Then, the pair correlation function can be estimated as:

$$g(r) = \sum_{i=1}^N \sum_{\substack{j=1 \\ i \neq j}}^N \frac{w(r_{ij} - r)}{\lambda^2 2\pi r s(r)}$$

where $w(\cdot)$ is the Epanechnikov kernel function defined above, $\lambda = N/A$ is the estimated density (gold-dots per unit area), r_{ij} is the distance between the gold-dots i and j and $s(r)$ is the edge correction factor. For rectangular or square plots, the Ohser-Stoyan edge correction factor [9] can be adopted:

$$s(r) = b_1 b_2 - \frac{r}{\pi} (2b_1 + 2b_2 - r), \text{ for } 0 < r < b_1 \leq b_2$$

where b_1 and b_2 are the side lengths of the picture (that means the height

and the width) and $s(r) < A = \text{width} \times \text{height}$.

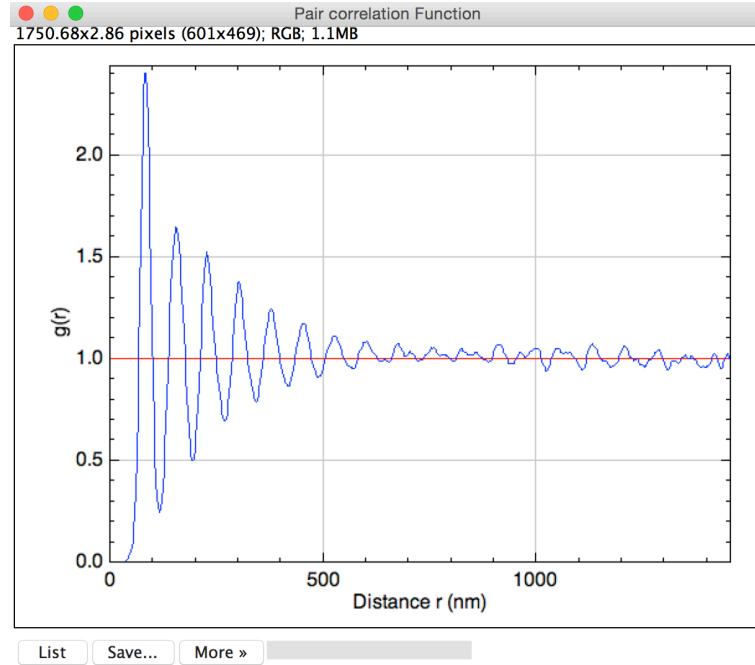


Fig. 7: Pair Correlation Function $g(r)$ of Fig. 1. In red $g(r)=1$ corresponds to a homogeneous Poisson process.

10) "Bond-orientational correlation function" (Fig. 8):

The (global) bond-orientationel order parameter Φ_6 was introduced by D. R. Nelson and B. I. Halperin to characterize the structural order in 2D systems [10-11]. It is given:

$$\Phi_6 = \left| \frac{1}{N} \sum_{i=1}^N \psi_6(\mathbf{r}_i) \right|$$

with ψ_6 is the local value for the particle i located at $\mathbf{r}=(x,y)$:

$$\psi_6(\mathbf{r}_i) = \frac{1}{n_i} \sum_{j=1}^{n_i} e^{6I\theta_{ij}}$$

where I is the imaginary unit ($I^2=-1$), θ_{ij} is the angle between the particles i and j and an arbitrary but fixed reference axis and n_i the number of nearest neighbors of the dot i . The bond-orientational correlation length ξ_0 was extracted from the "zero-momentum" correlation function of ψ_6 which is called bond-orientational correlation function $g_6(r)$ and defined by:

$$g_6(r) = \frac{\langle \psi_6^*(\mathbf{r}') \psi_6(\mathbf{r}' - \mathbf{r}) \rangle}{\langle \delta(\mathbf{r}') \delta(\mathbf{r}' - \mathbf{r}) \rangle} = \frac{\left\langle \sum_i \sum_{i < j} \psi_6^*(\mathbf{r}_i) \psi_6(\mathbf{r}_j) \delta(\mathbf{r} - (\mathbf{r}_i - \mathbf{r}_j)) \right\rangle}{g(r)}$$

where the denominator is related to the pair correlation function $g(r)$ and:

$$\sum_i \sum_{i < j} = \sum_{i=0}^{N-1} \sum_{j=i+1}^N$$

For a 2D system (or the quasi long-ranged bond orientational order of the hexatic state), the envelope of this correlation function decay to zero exponentially [12-14]:

$$g_6(r) \propto \exp\left(-\frac{r}{\xi_0}\right)$$

Hence, ξ_0 is a measure for the typical size of the single crystalline domain, i.e., larger is ξ_0 and larger is the crystalline domain (and better is the order). The bond-orientational correlation length ξ_0 is determined by fitting $\log(g_6(r))$ with a line $A + B$ by using the fitting algorithm described in Numerical Recipes 15.2. The plugin shows the function $g_6(r)$ with the exponential fit in red and the length ξ_0 (with the χ^2 test) in the same plot (Fig. 8).

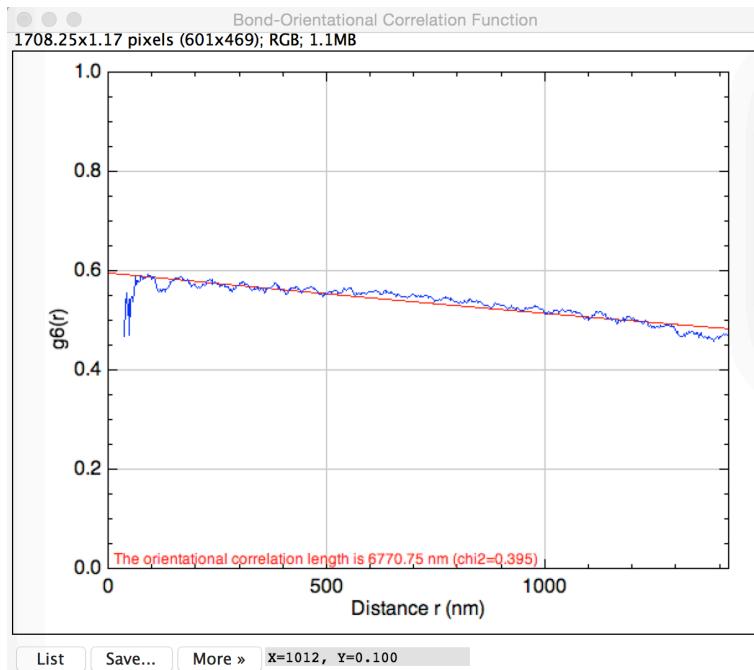


Fig. 8: Bond-orientational Correlation Function $g_6(r)$ of Fig. 1. In red is the fit of $g_6(r)$ with an exponential decay.

- 11) "Save Spacing and Order in a table": this indicates that you want to save the spacing and the order in a text file. If you select this option, a "Save Spacing & Order" window will appear at the end of the analysis (see below).

When you have finished, press OK

3. Analysis

At the beginning of the process,

First, the image is converted to a binary image to reveal the spots (by the threshold method you have selected) and an overlay of the image with the subtracted background (in grey) and the threshold image (in red) is shown in order to select a rectangular ROI (in yellow) with the “Rectangle Tool” of the Toolbar to remove the text of the micrograph or the area where the plugin is not able to detect simple spots but huge multiple spot, or where there is some disinclinations. For example in the figure below, a rectangular area (in yellow) that removes the bottom part is selected (Fig 9, left). After selection, you can click OK on the frame that appears at right of the overlay. If you want to select the full image, just press ok without selection (Fig. 9, right).

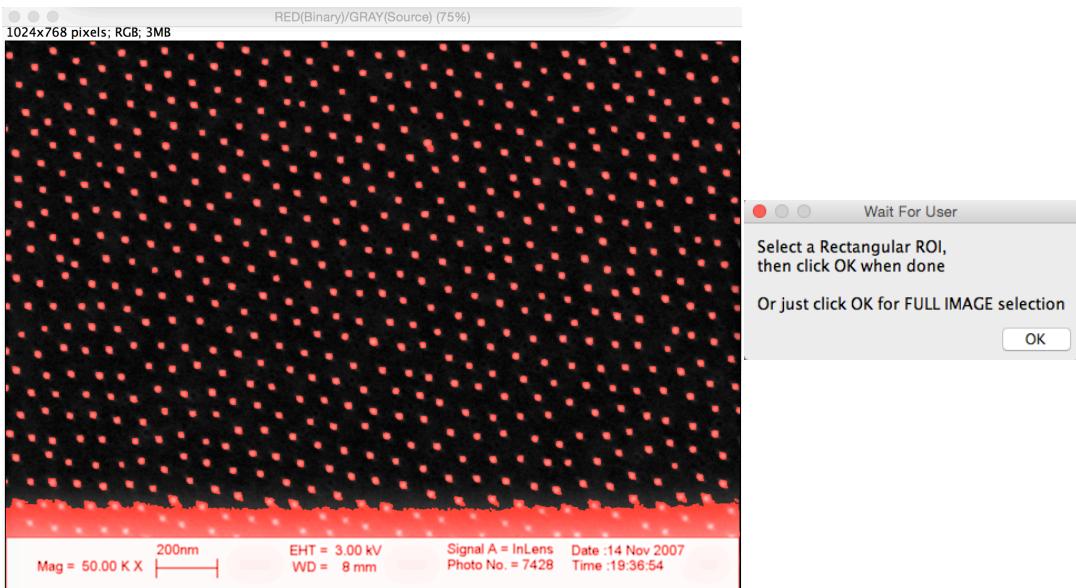


Fig. 9: The Overlay: Subtracted-background image (in grey) / threshold image (in red)

Then, the function “ParticleAnalyzer” that you can found directly under “Analyze/Analyze Particles...” is applied to detect the position of the different spots (the parameter “Min size” you have selected at the beginning is used here to remove all the spot below this size in pixel²). This process permits to measure the centre of mass of each spot.

At the end of the process,

The “Results” main window gives you the results of the 6 closest-neighbor method (Fig 10).

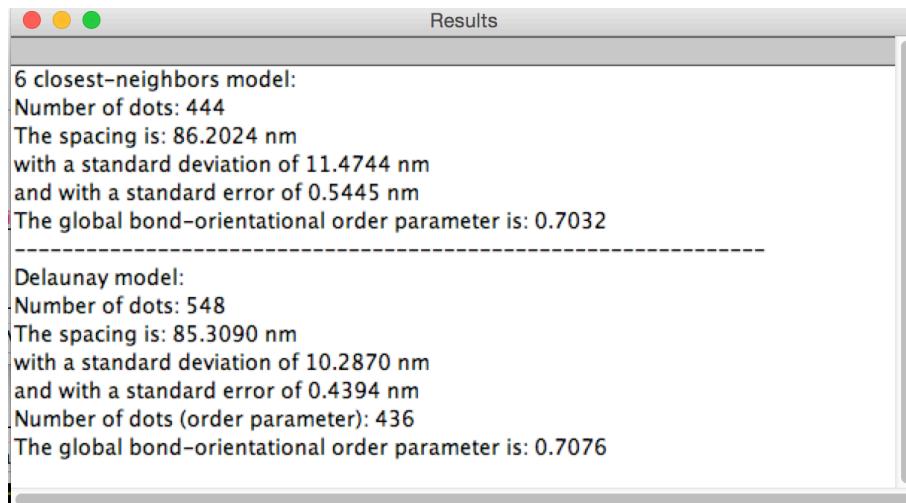


Fig. 10: The "Results" main window: Analysis of the selected region (of Fig. 9).

You can also restart the process if you did a mistake by clicking "Yes, please", or press "No way!" if you want to continue (Fig. 11).

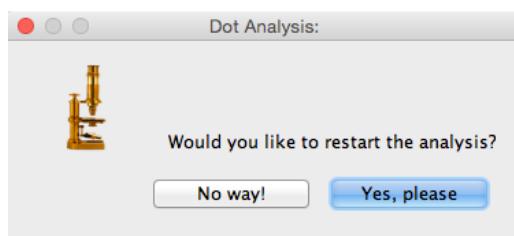


Fig. 11: Restart or not Window.

4. Save Result: Spacing and Order

You have to fill the different characteristics of your system in the "Save Spacing & Order" main window (Fig. 12) and all these parameters are saved in a new or existed file (see Fig 13).

- First row: Polymer
 - First copolymer (in the list: PS, P2VP, PDMS, PMMA) with the number of monomer (Fig. 12a at left)
 - Second copolymer (Fig. 12a at right)
- Second row:
 - Loading rate of gold (between 0 and 1),
 - Polymer concentration (in mg/ml)
- Third row:
 - Select if you have use the dipping method (False) or spinning method (True). If you select spinning method the "dipping speed" is disabled and in grey because you do not have to specify it (Fig. 12b).
 - Dipping speed (in Volts).
- Fourth row:
 - Date of the file when it was created (automatically filled with the information of the image),

- Add in an existed file (Yes = True, No = False). The created file is automatically saved in the folder of the analysed image.

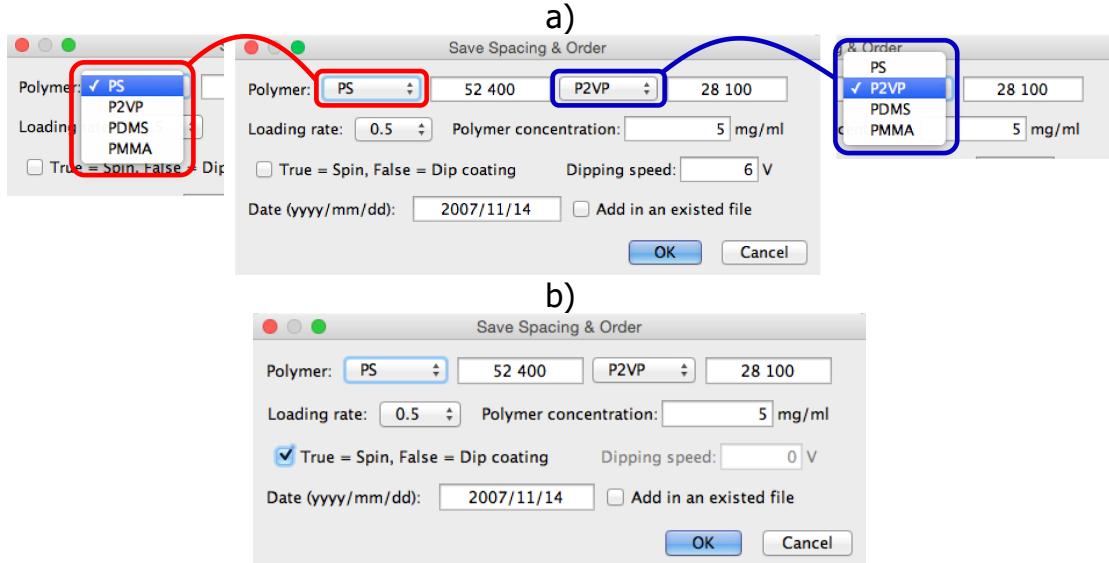


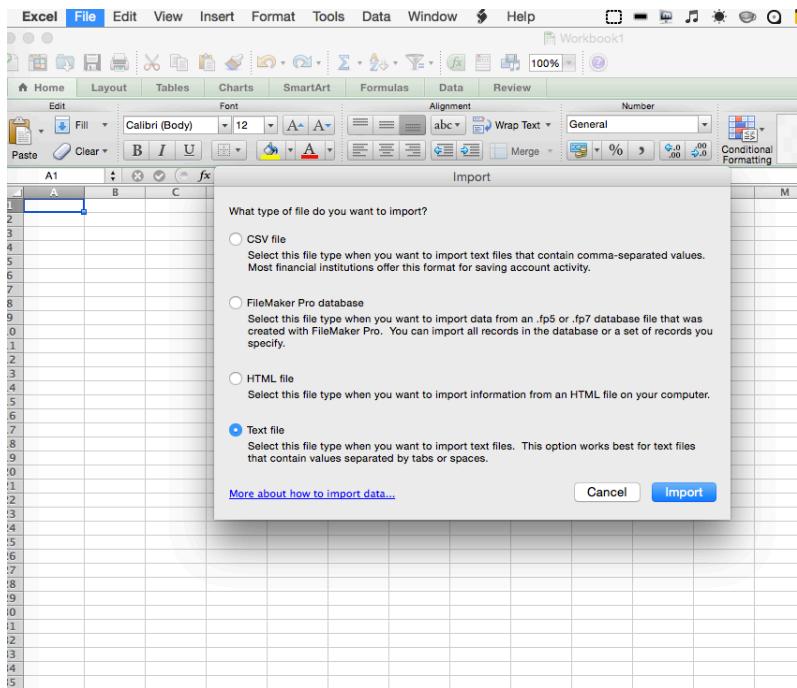
Fig. 12: The "Save Spacing & Order" main window.

The File created contains the following parameters of the analysis:

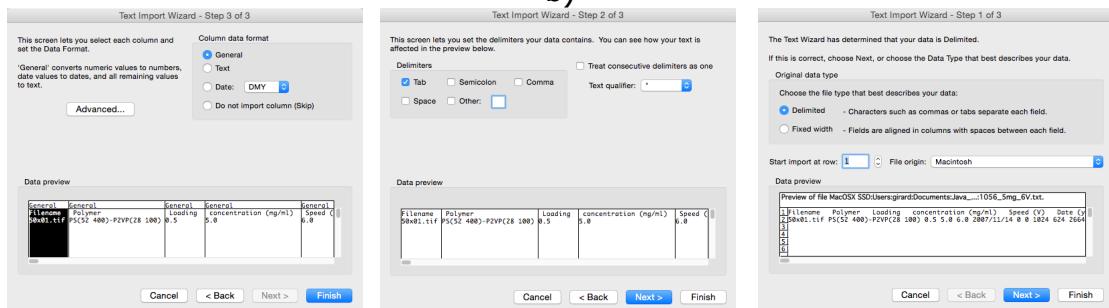
- 1) Filename,
- 2) Polymer, (example: PS[52400]-P2VP[28100])
- 3) Loading,
- 4) Concentration (mg/ml),
- 5) Speed (V),
- 6) Date (yy/mm/dd),
- 7) Region of Interest: X, Y, Width, Height (a column for each parameter)
- 8) Number of dots (with the 6-nearest neighbor model),
- 9) Spacing in nm
- 10) Stdev in nm (Standard deviation)
- 11) Serror in nm (Standard error)
- 12) Order Parameter
- 13) Number of dots (with the Voronoi/Delaunay model)
- 14) Spacing in nm (with the Voronoi/Delaunay model)
- 15) Stdev in nm (with the Voronoi/Delaunay model)
- 16) Serror in nm (with the Voronoi/Delaunay model)
- 17) Order Parameter (with the Voronoi/Delaunay model)

To open the file you can use a spreadsheet software or Excel (with File▷ Import) as indicated in Fig. 13:

a)



b)



c)

	Filename	Polymer	Loading	concentration (mg/ml)	Speed (Date (mm/dd)	Roi X (pixel)	Roi Y (pixel)	Roi Width (pixel)	Roi Height (pixel)	Number of dots	Spacing (nm)	StdDev (nm)	Order	Number of dots (Delaunay)	Delaunay Sp (nm)	Delaunay St Dev (nm)
1	50x01.tif	PS(S2 400)-PVPC(Z8 100)	0.5	5.0	6.0	14/11/07	0	0	1024	617	2622	86.21192452	0.54657638	0.70470013	85.31950698	10.35064917	0.44709588
2	50w01.tif	PS(S2 400)-PVPC(Z8 100)	0.5	5	6												

Fig. 13: In Excel use File/Import and select "Text file" (panel a). Text Import Wizard in 3 steps from Excel(panel b). Panel c is the result of the imported file corresponding to the parameters of the analysed image (Fig. 9).

References

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