

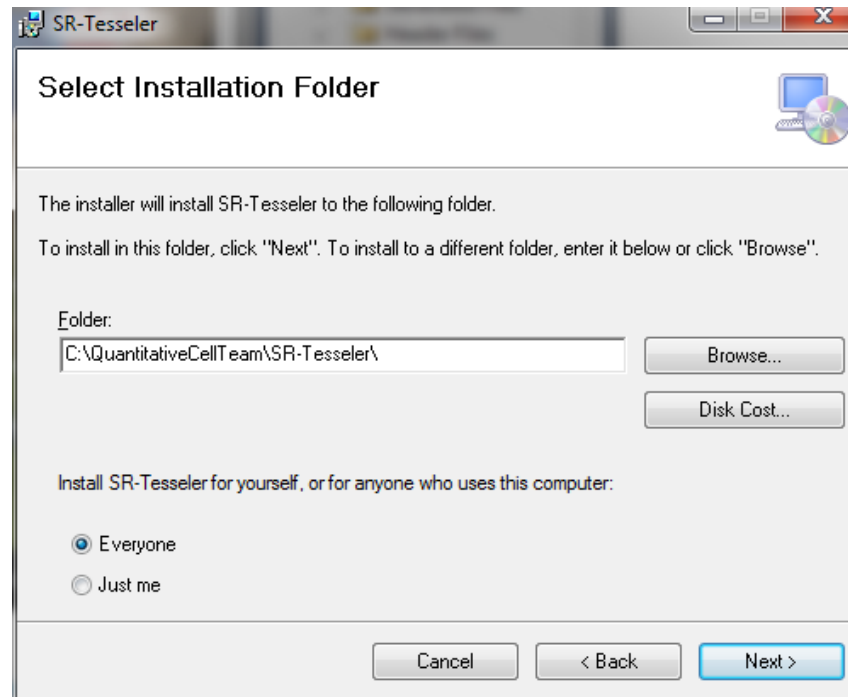
SR-TESSLER

Installation guide & Manual

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CNRS UMR5297, Univ. Bordeaux

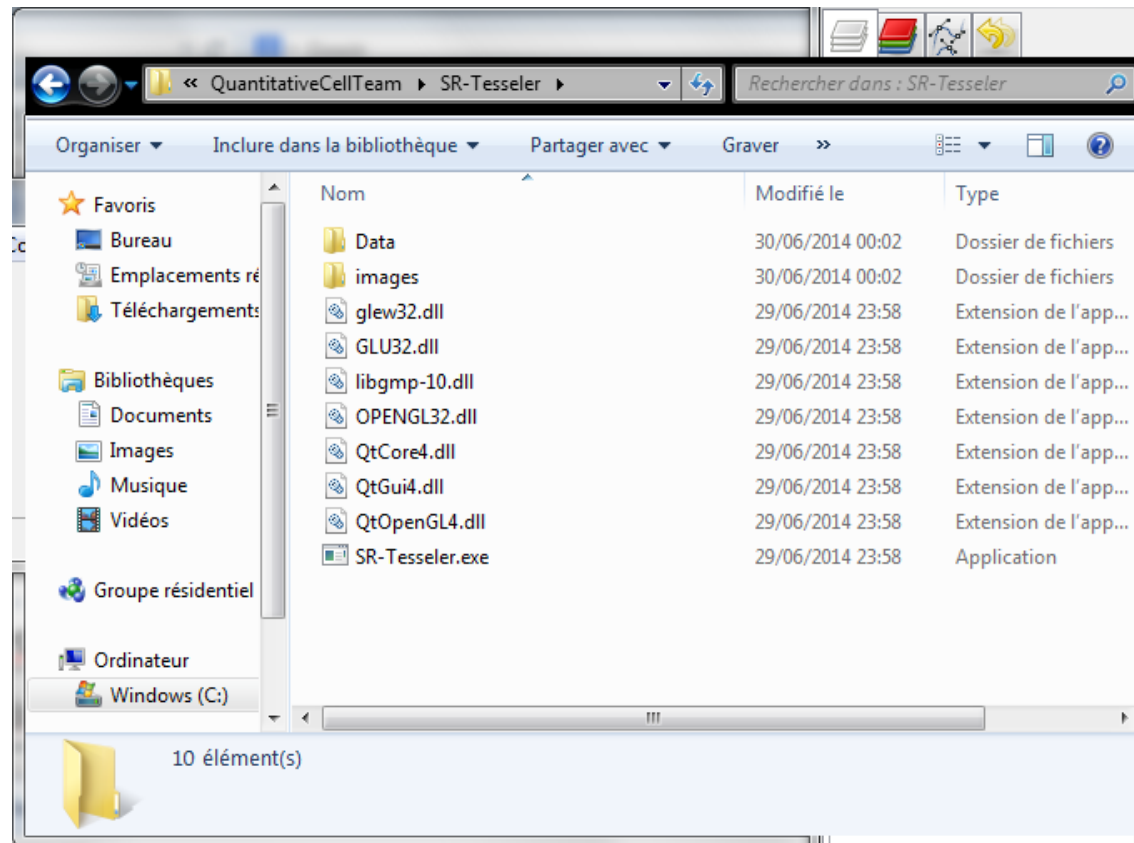
Installation (1/3)

- Need a PC with OpenGL installed
- Launch « Setup.msi »
- Select installation directory



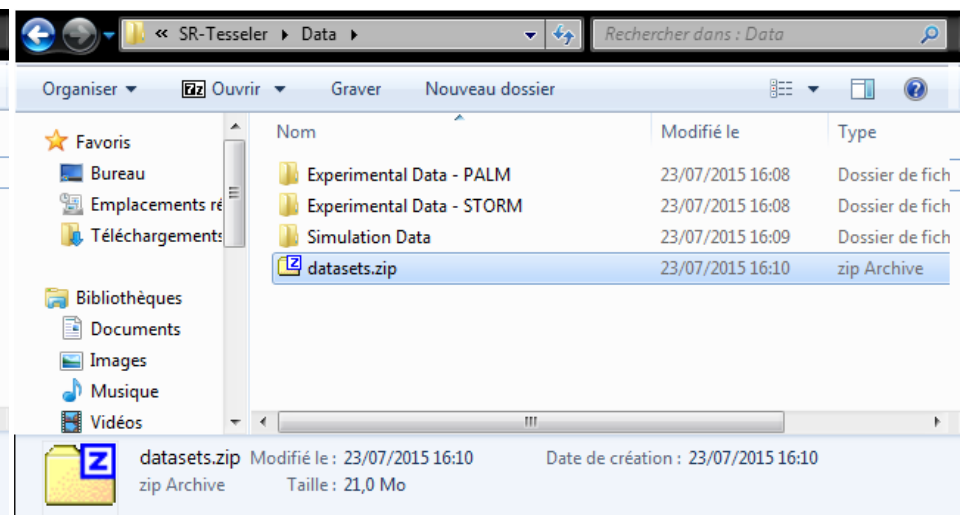
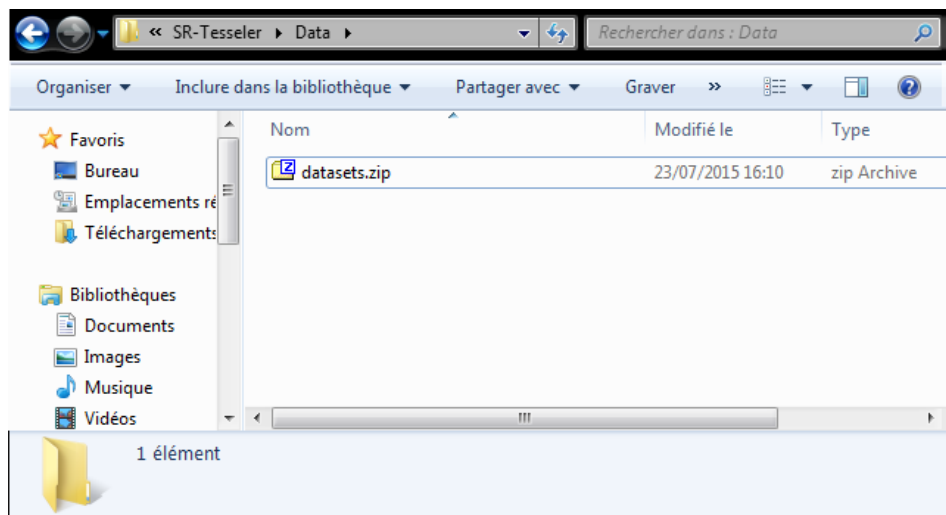
Installation (2/3)

- Installation folder contains executable, icons, DLLs files and dataset examples



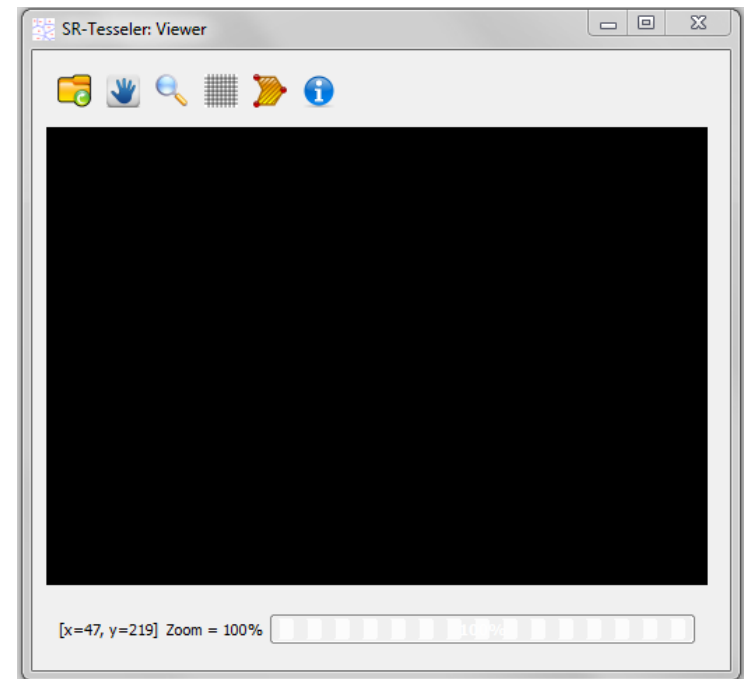
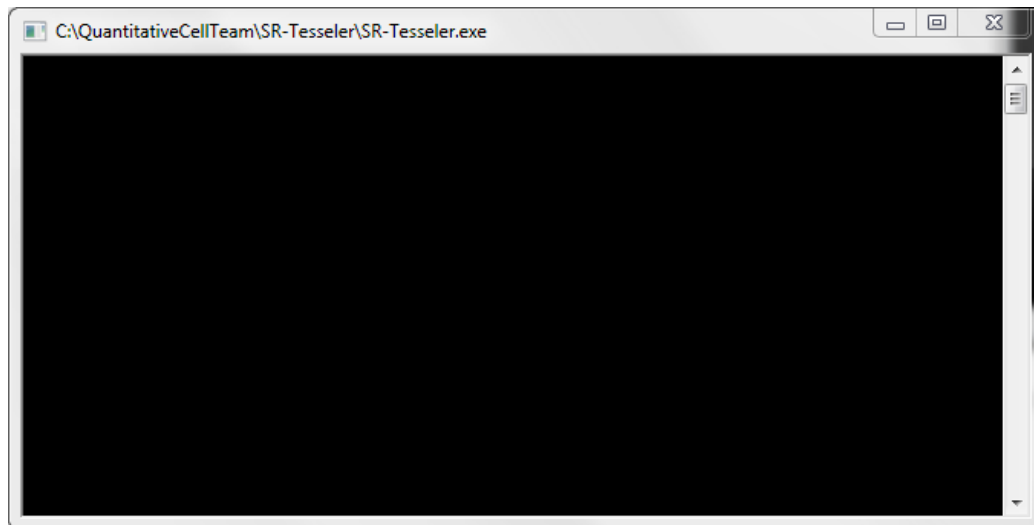
Installation (3/3)

- Unzip « datasets.zip » example files (in Data directory). This will create three sub-directories (Experimental Data – PALM, Experimental Data - STORM and Simulation Data)



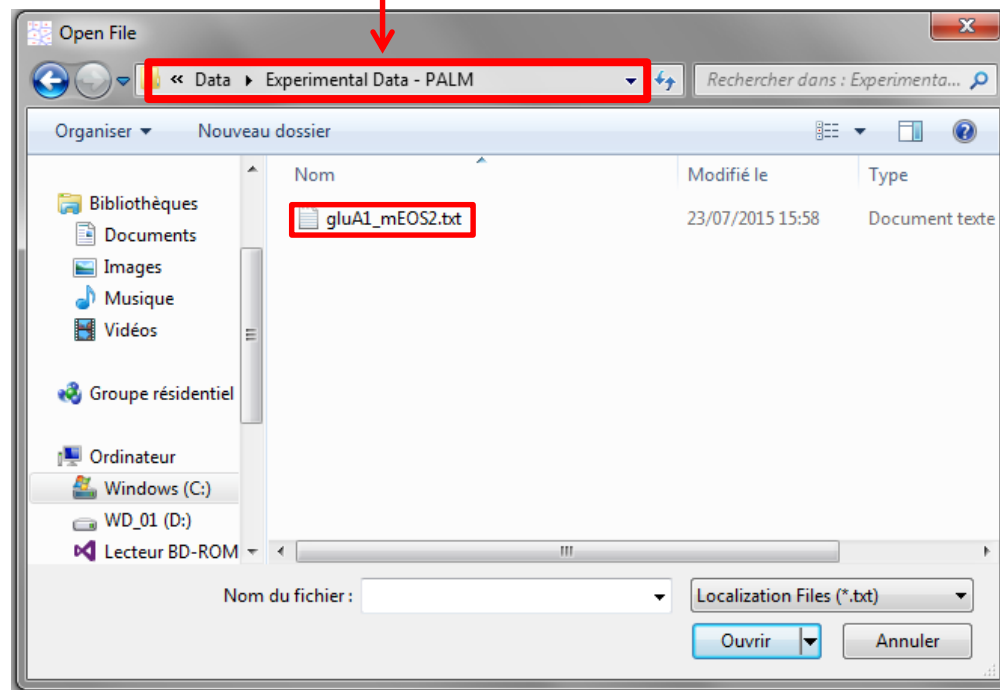
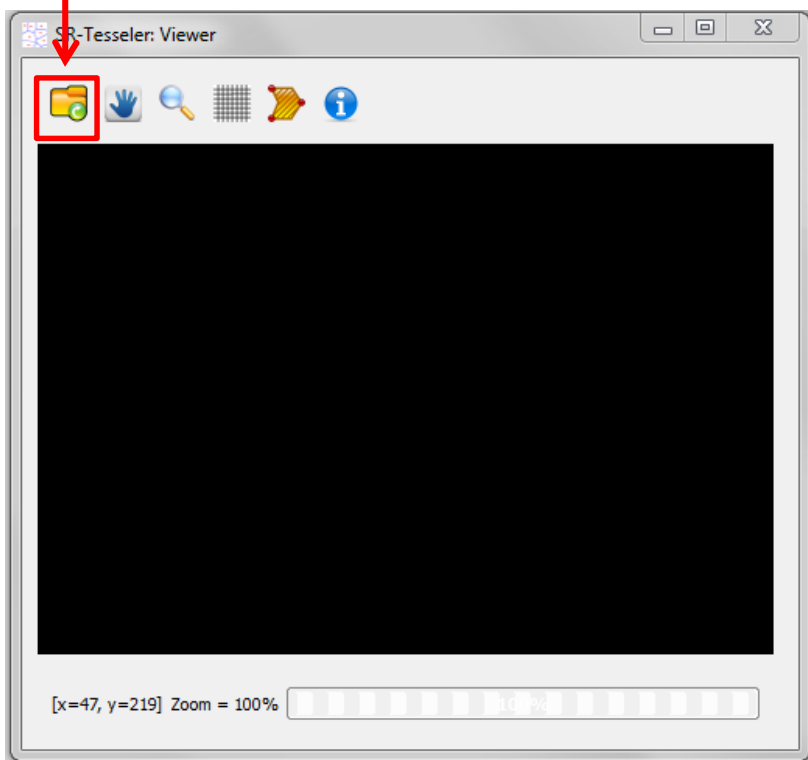
SR-Tesseler Quick start (1/2)

- Launch « SR-Tesseler.exe »
- 2 windows will pop-up
 - a Console (for application messages)
 - A Viewer



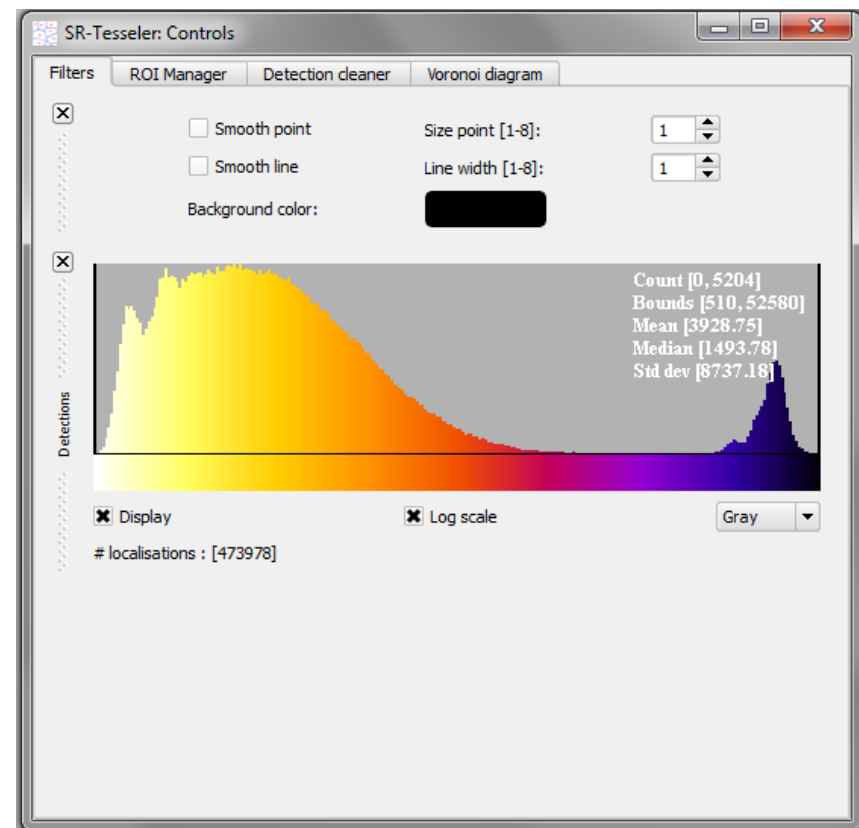
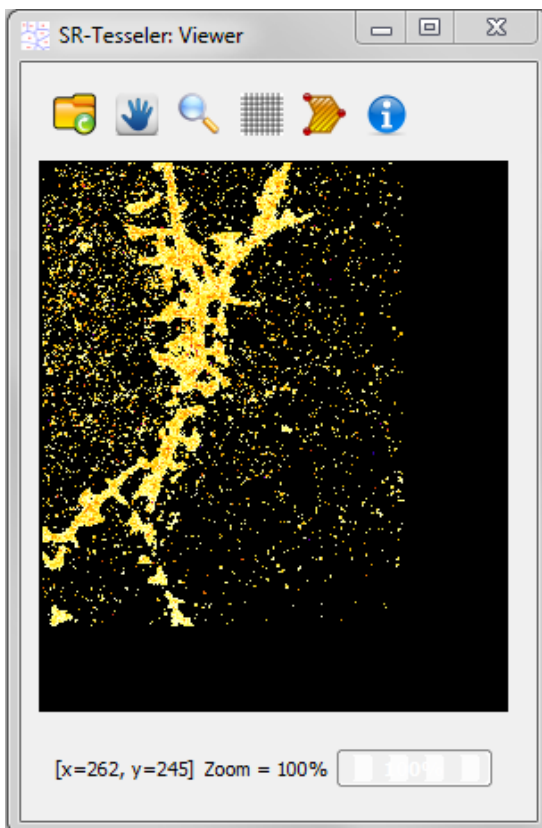
SR-Tesseler Quick start (2/2)

- To open single-molecule data:
 - Click on the open icon
 - Select localization file (e.g. « \Experimental Data - PALM\gluA1_mEOS2.txt »)



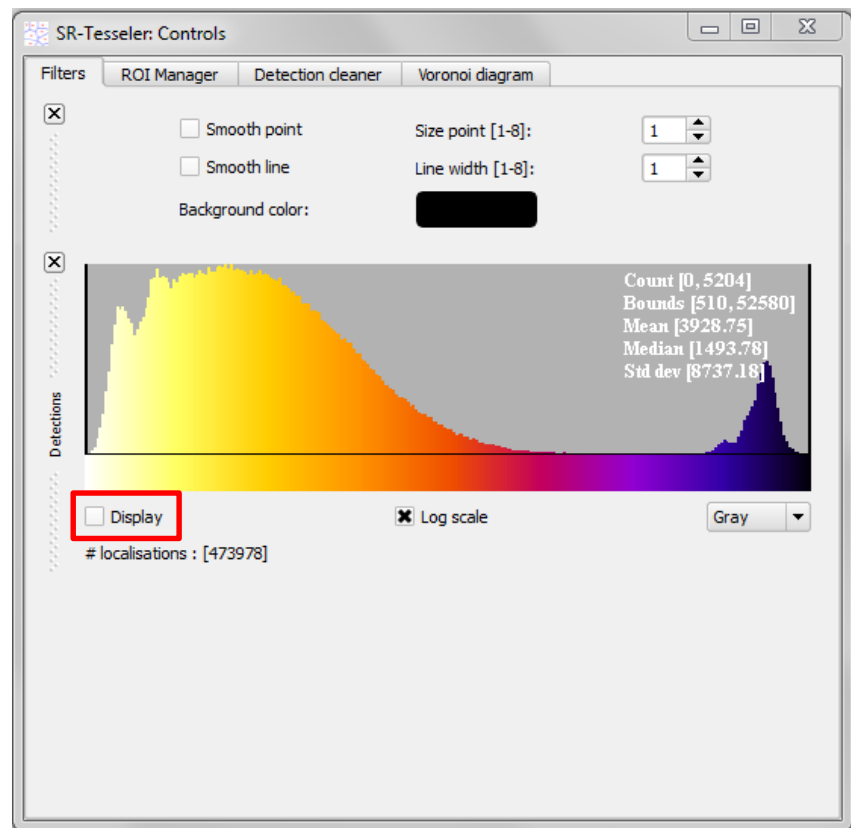
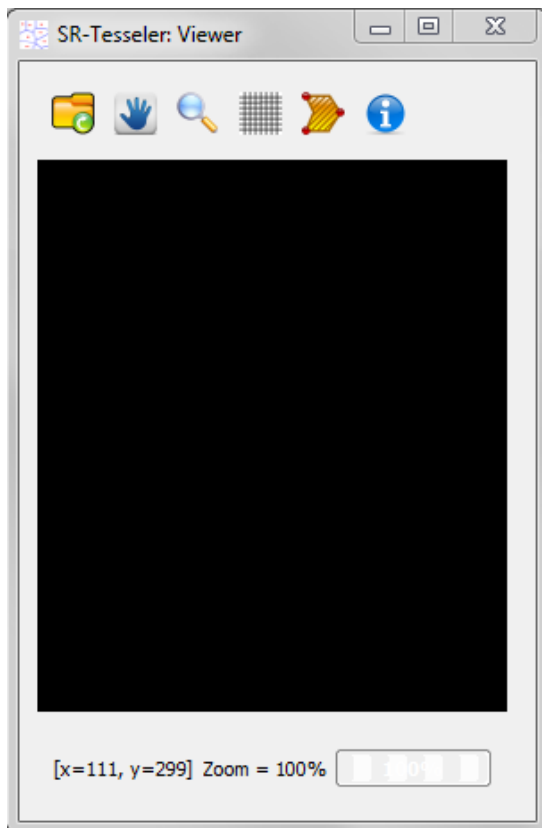
SR-Tesseler GUI (1/7)

- Once loaded:
 - Data are displayed in the SR-Tesseler Viewer window
 - An control window with histogram and options is displayed



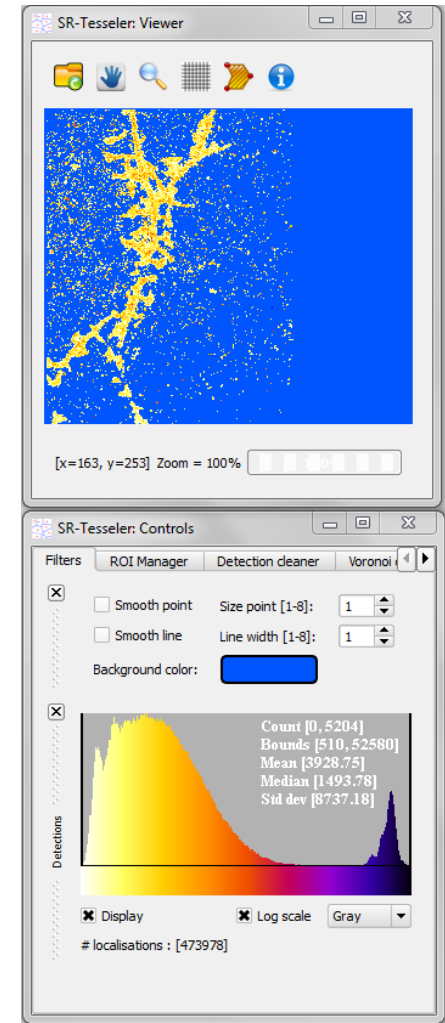
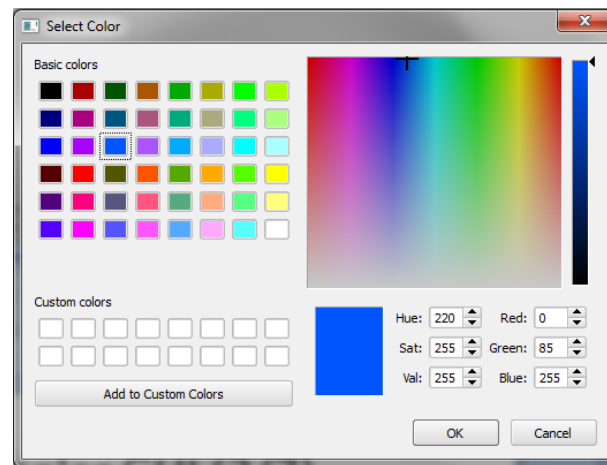
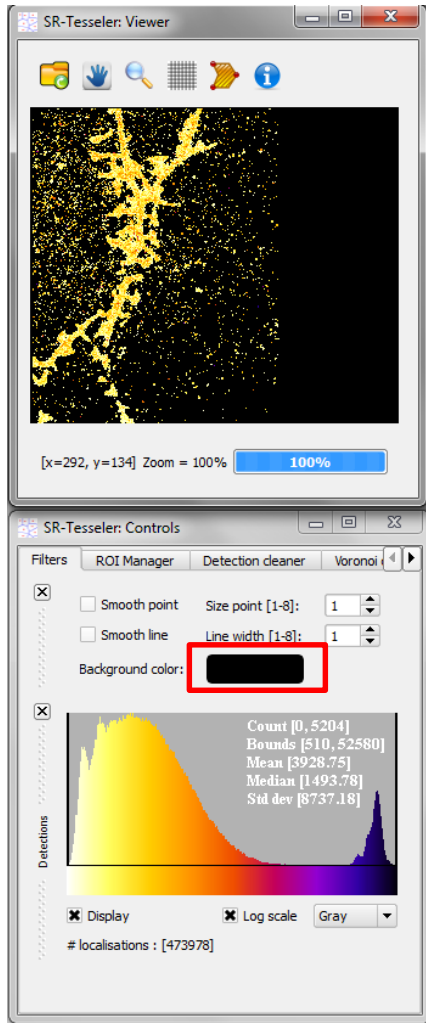
SR-Tesseler GUI (2/7)

- Single-molecule detections can be displayed by checking the « Display » check-boxes



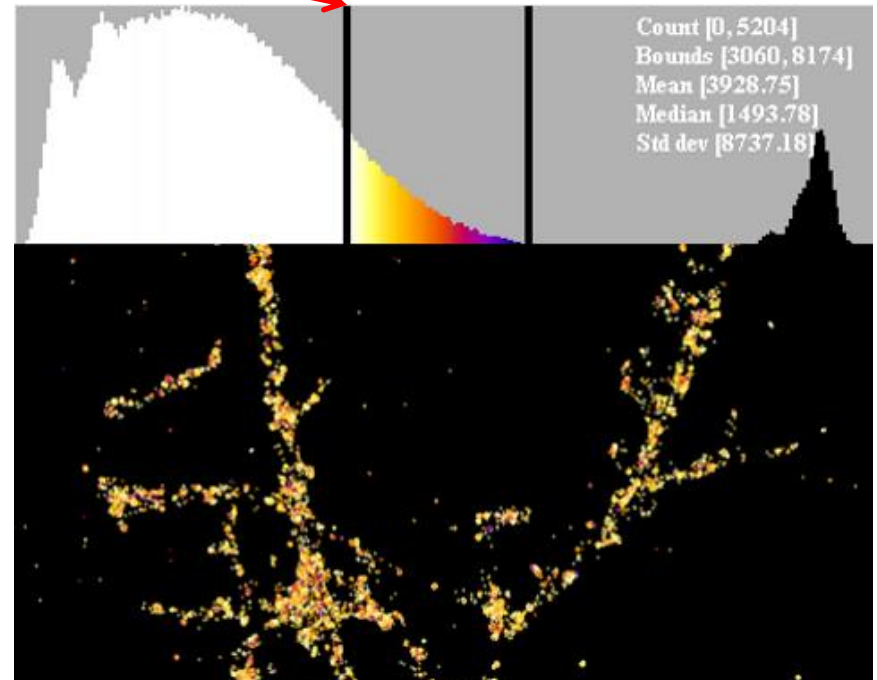
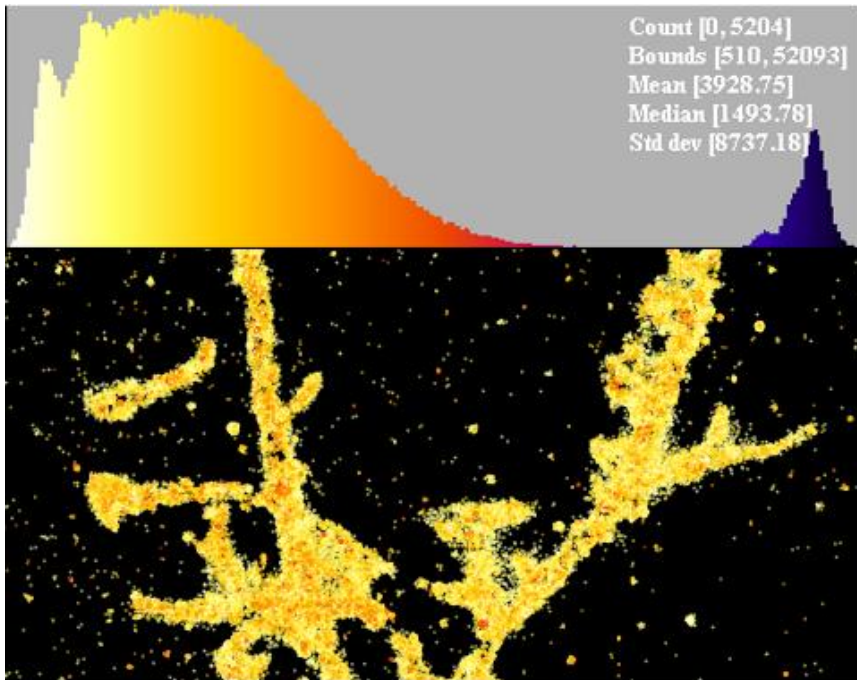
SR-Tesseler GUI (3/7)

- Click on the background color to change it



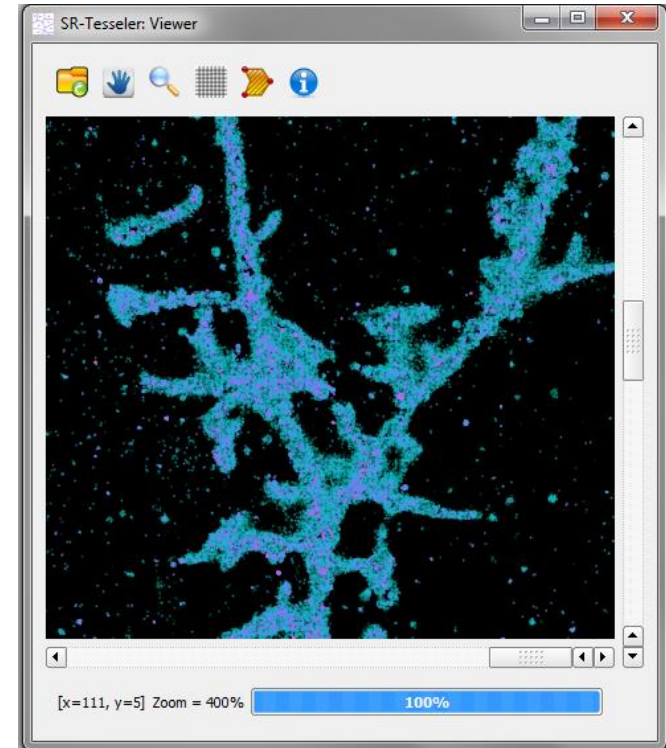
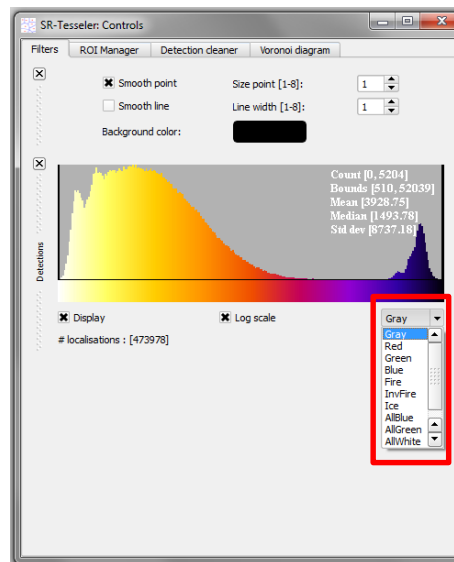
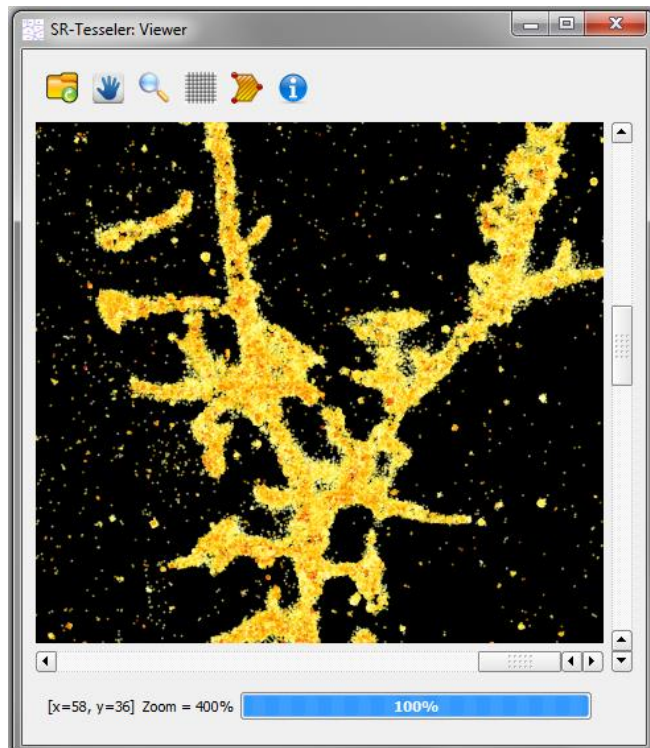
SR-Tesseler GUI (4/7)

- Intensity scaling is performed by clicking on the histogram: left (resp. right) selects for min (resp. max)



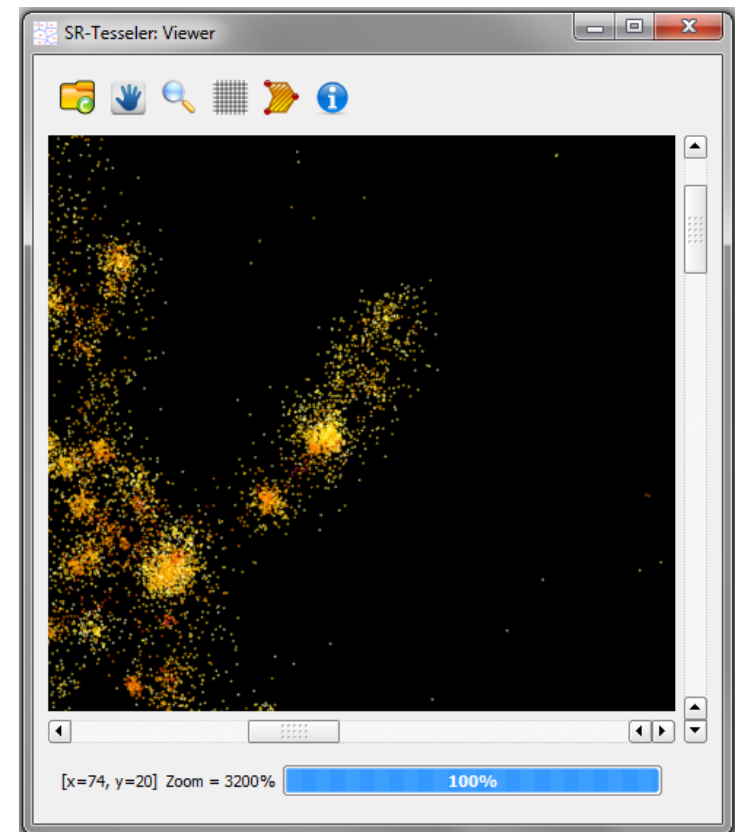
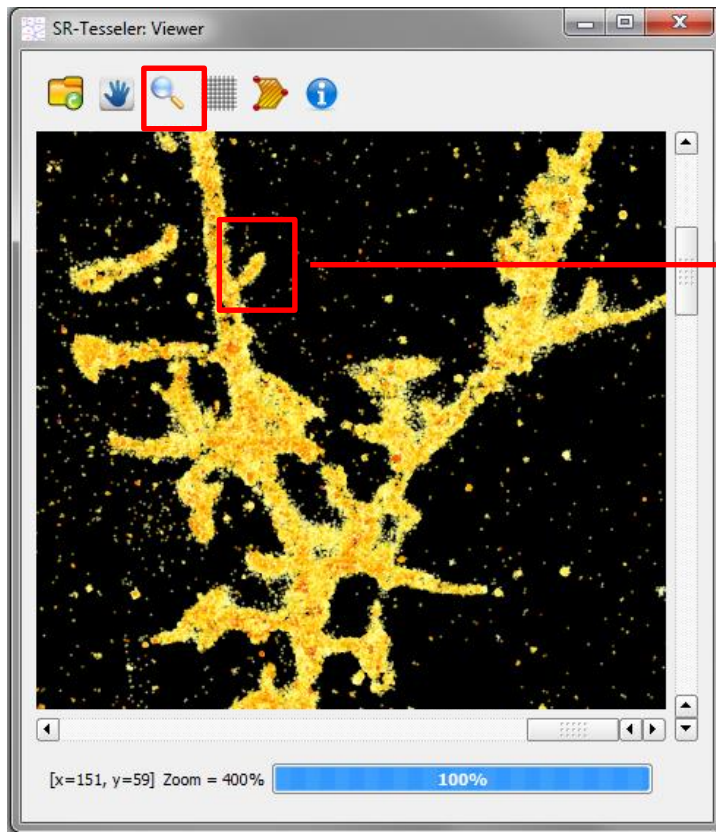
SR-Tesseler GUI (5/7)

- Change the LUT by clicking on the combo box.



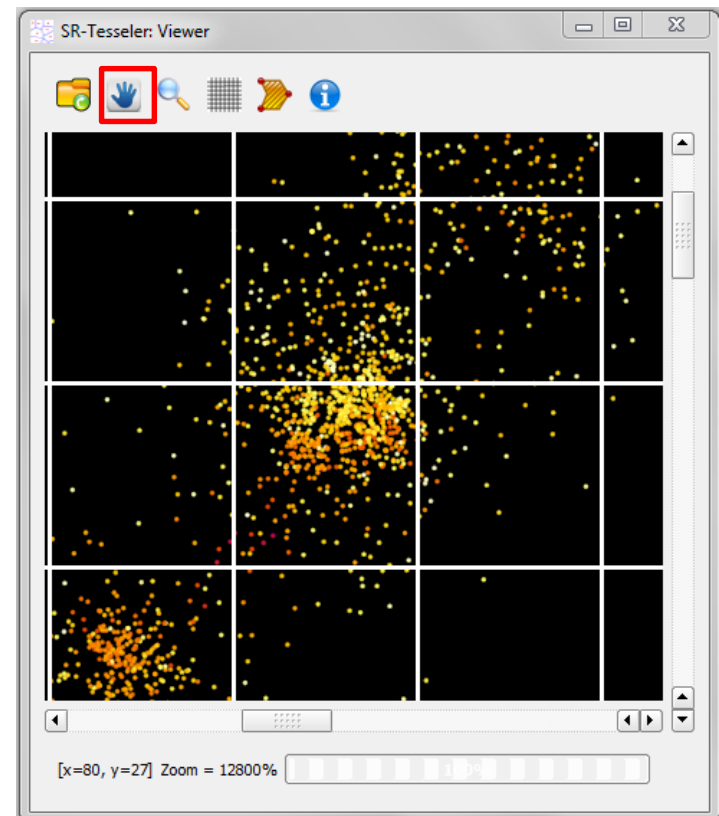
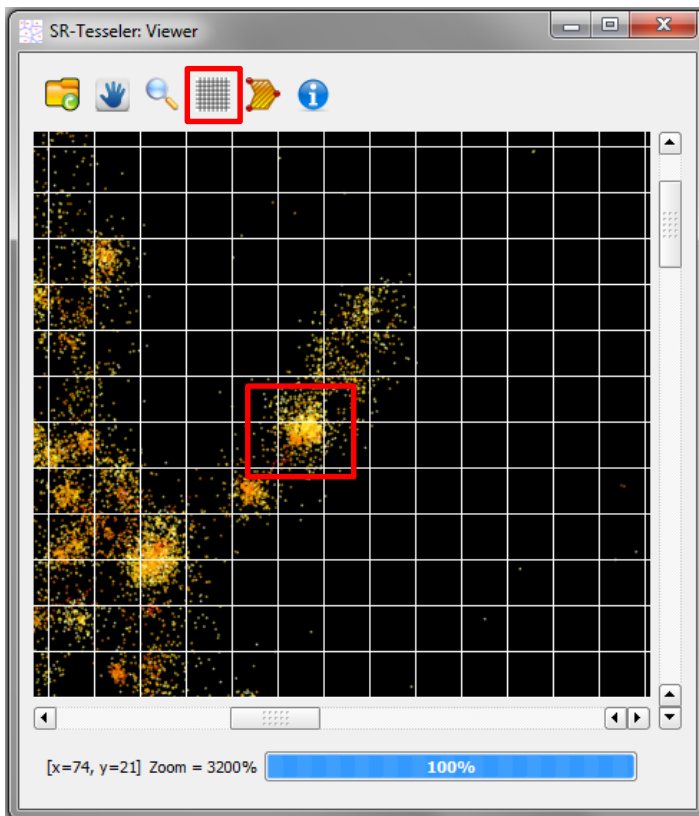
SR-Tesseler GUI (6/7)

- Zoom by clicking on the glass icon or scrolling the mouse wheel
- Then left (resp. right) click on the image to zoom in (resp. out)



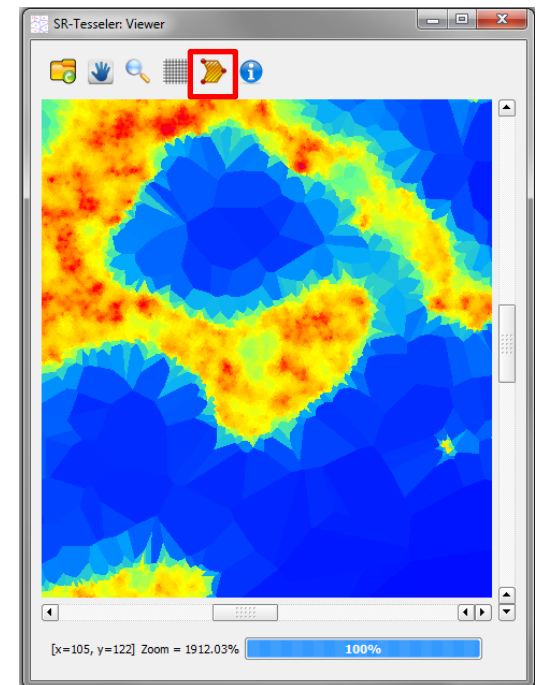
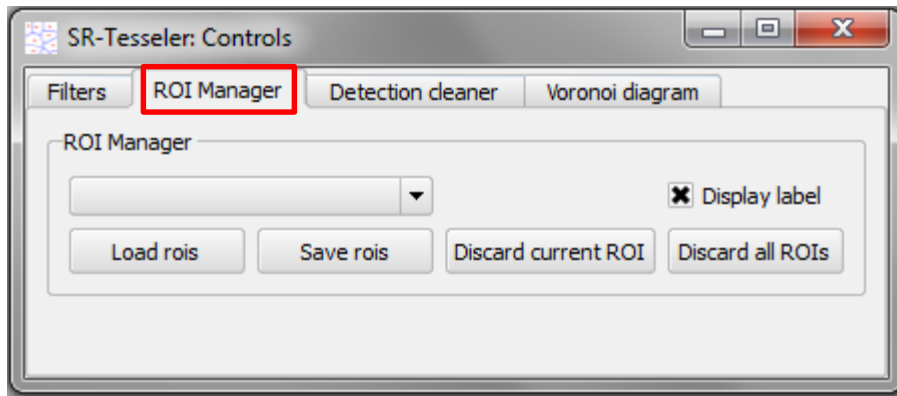
SR-Tesseler GUI (7/7)

- Select the grid icon to display a grid corresponding to low-resolution pixel size
- Select the hand icon to scroll the image



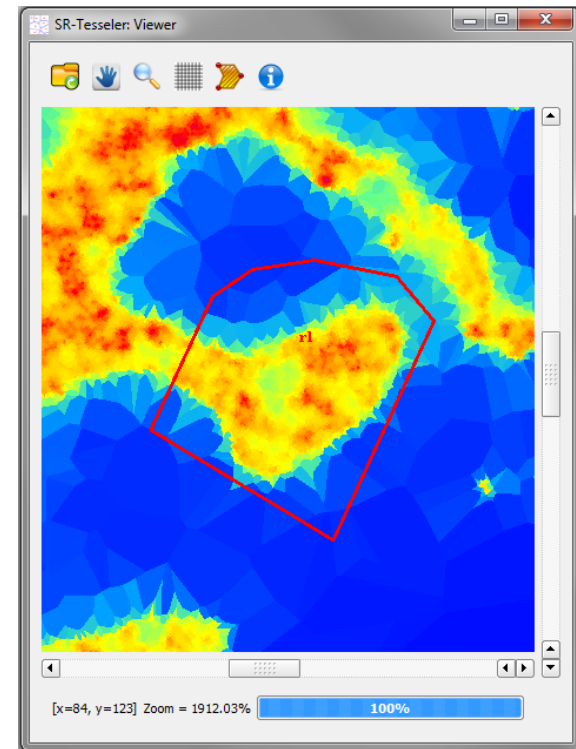
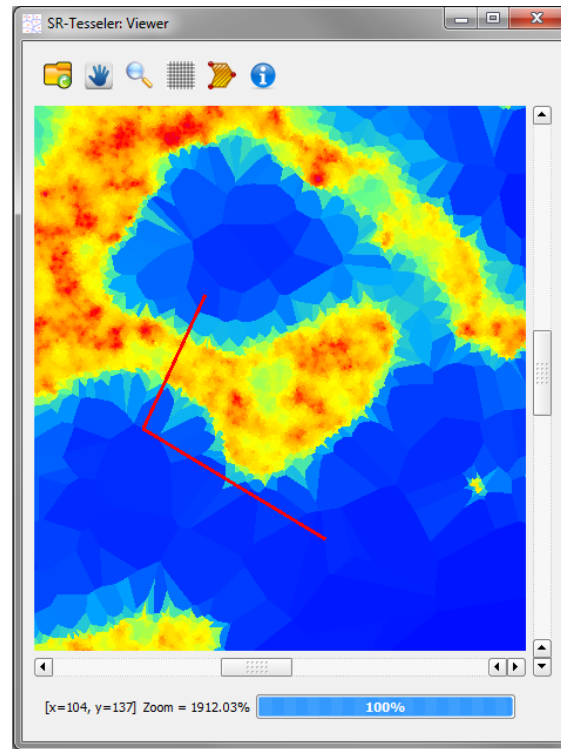
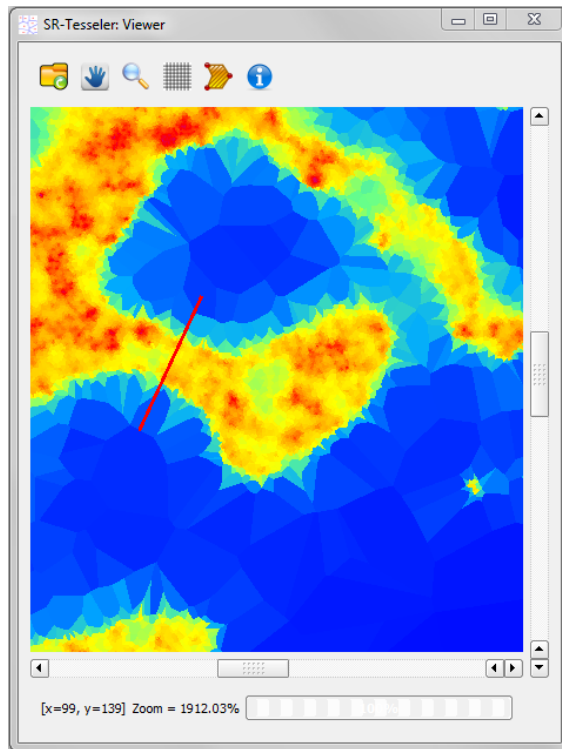
SR-Tesseler: ROI Manager (1/3)

- Select the « ROI Manager » tab in the control window
- ROIs can be used during the object and cluster creation
- To add a ROI, click on the Polygon icon



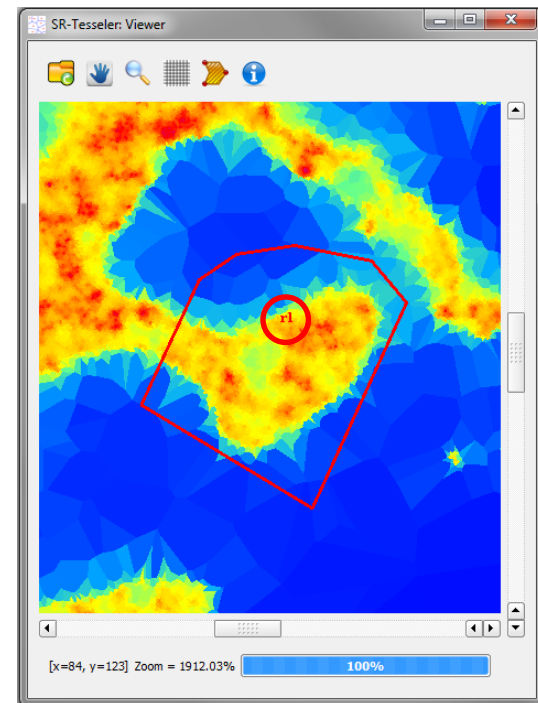
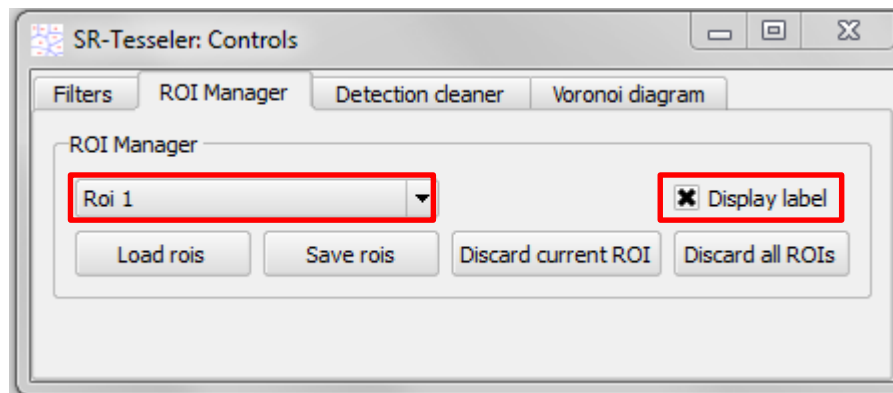
SR-Tesseler: ROI Manager (2/3)

- Left click will add a new point to the current ROI
- Double left click will end the current ROI



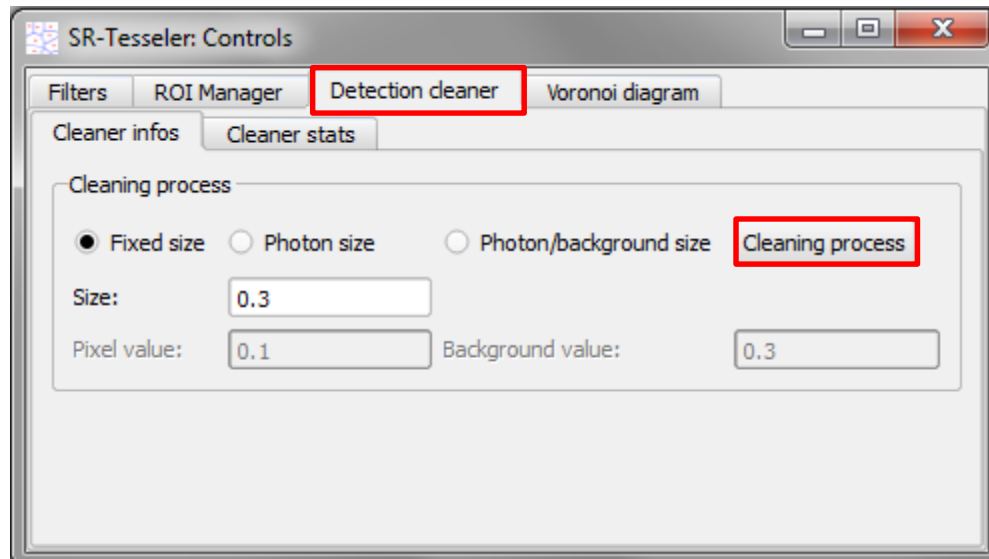
SR-Tesseler: ROI Manager (3/3)

- When a ROI is done, it is added to the ROI list
- Click on « Display label » to toggle the label display



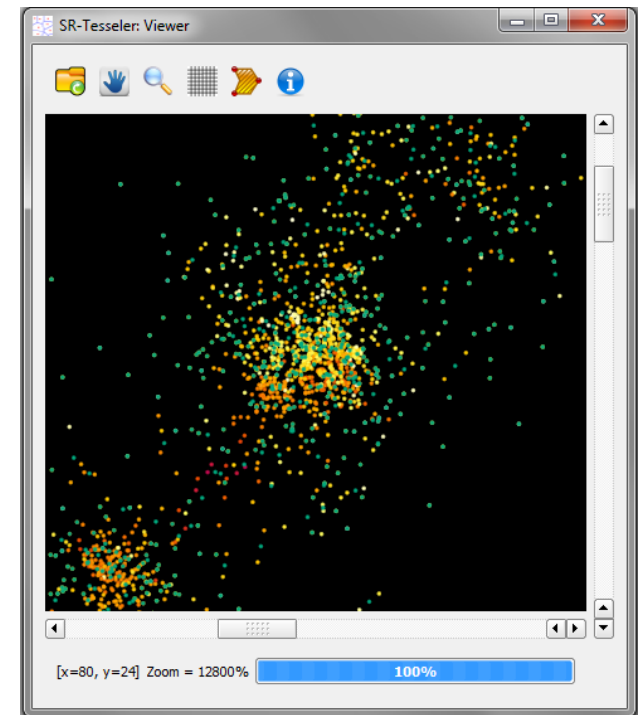
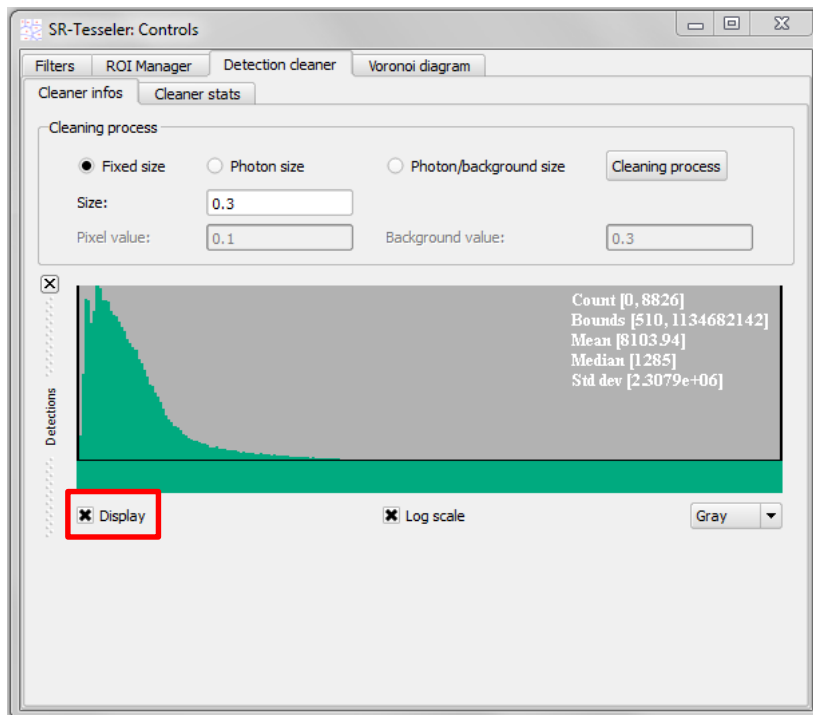
SR-Tesseler: blinking correction (1/4)

- Select the « Detection cleaner » tab in the control window
- Search radius can be fixed (in pix), photon or photon and background dependant
- Click the « Cleaning process » button



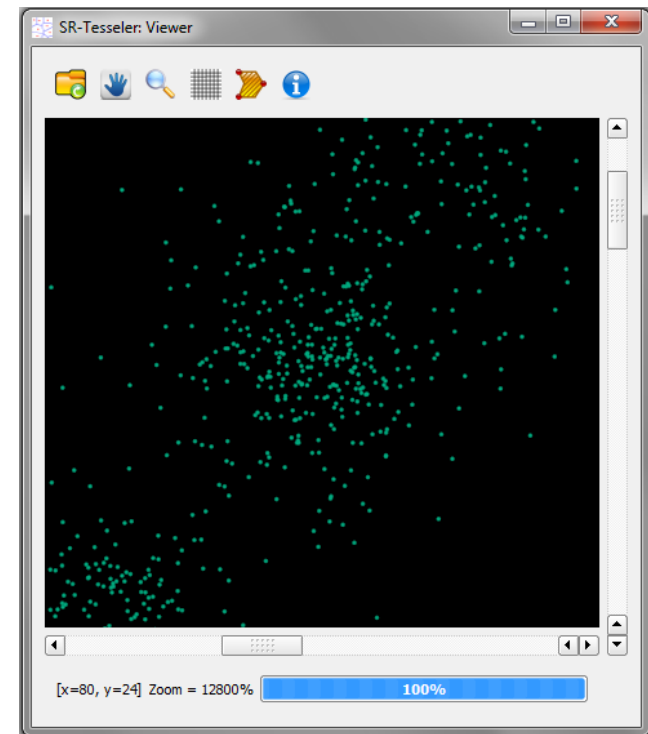
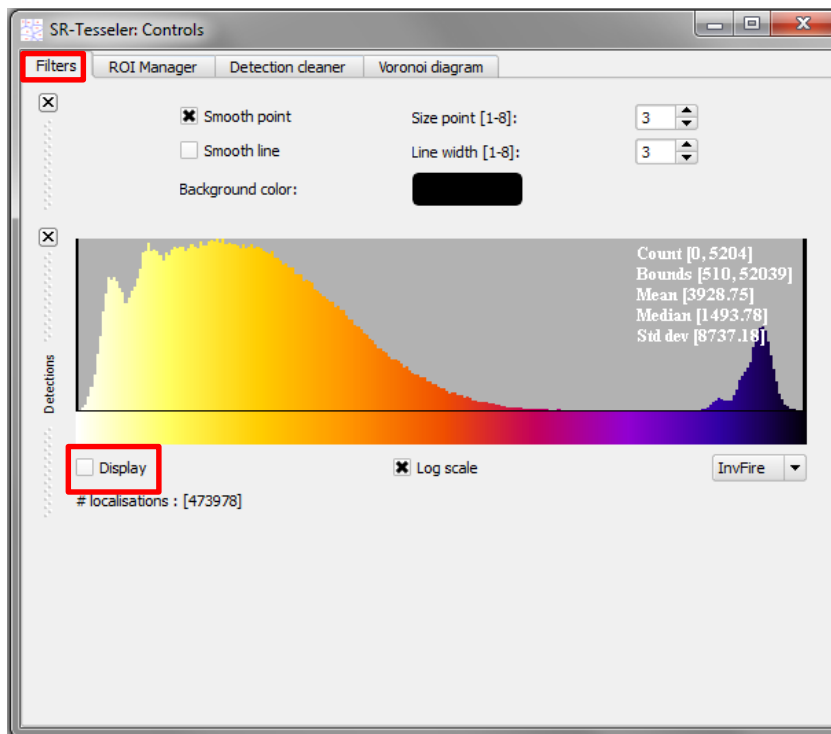
SR-Tesseler: blinking correction (2/4)

- The blinking-corrected detection dataset is created
- The two detection sets, initial and corrected, can be displayed by checking the Display checkboxes



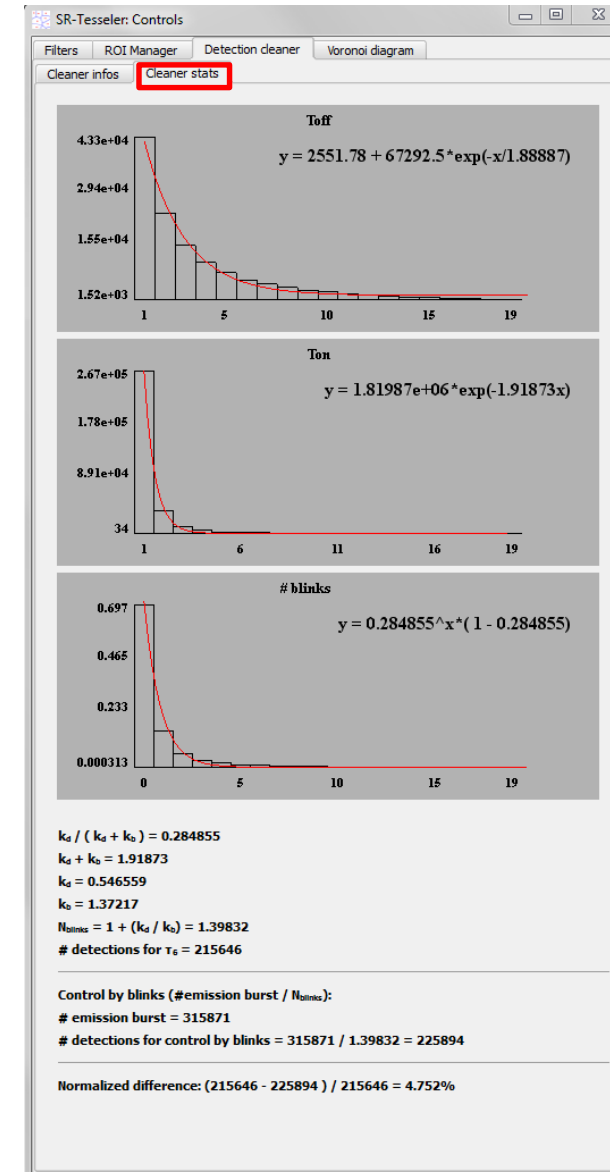
SR-Tesseler: blinking correction (3/4)

- Original dataset can be hidden by unchecking the « Display » checkbox in the « Filters tab »



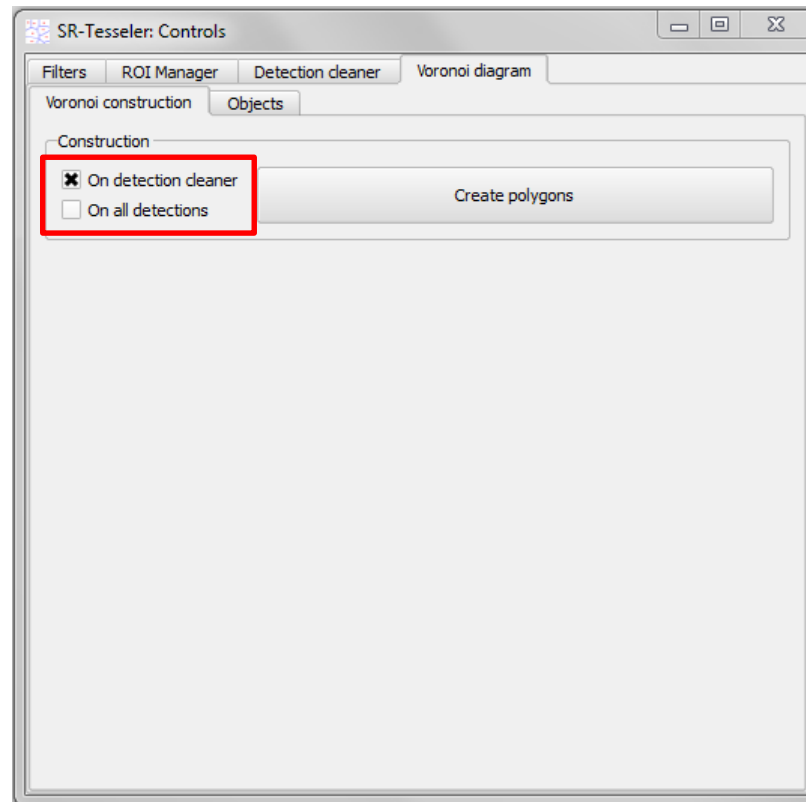
SR-Tesseler: blinking correction (4/4)

- All correction information and blinking statistics are displayed in the « Cleaner stats » tab
- *NB: Blinking correction doesn't work on the provided simulation data since all localizations were defined at the same time*



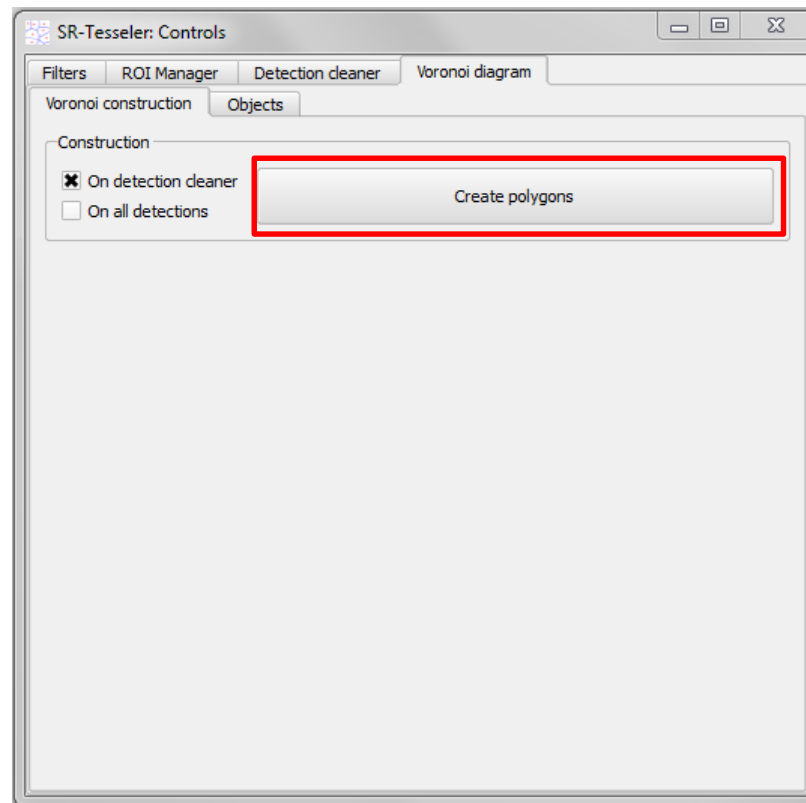
SR-Tesseler: Voronoï diagram (1/3)

- The Voronoï diagram can be computed either on the initial detection dataset, or on the blinking-corrected one (if exists)



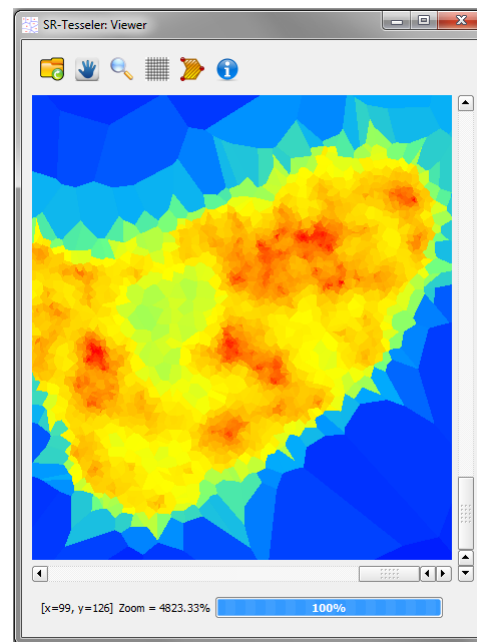
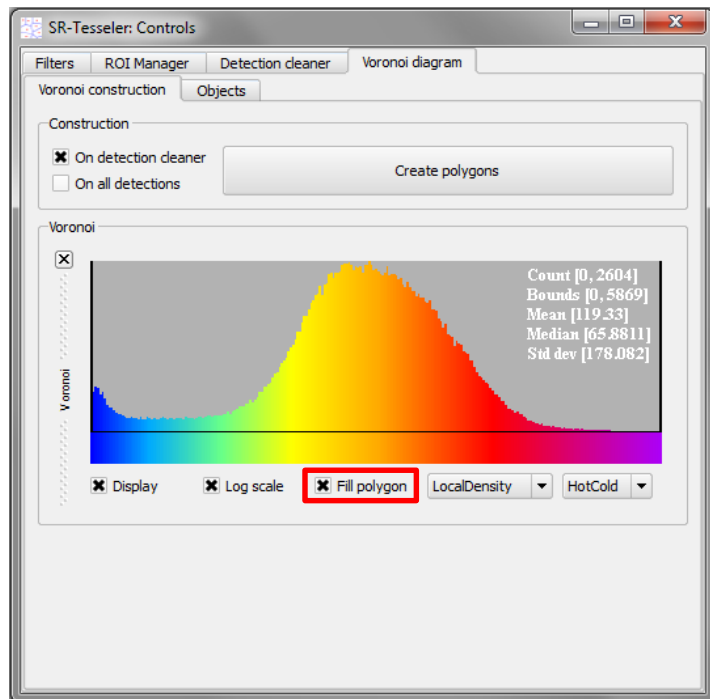
SR-Tesseler: Voronoï diagram (2/3)

- Click the « Create polygons » button from the « Voronoï diagram » tab to generate the tessellation

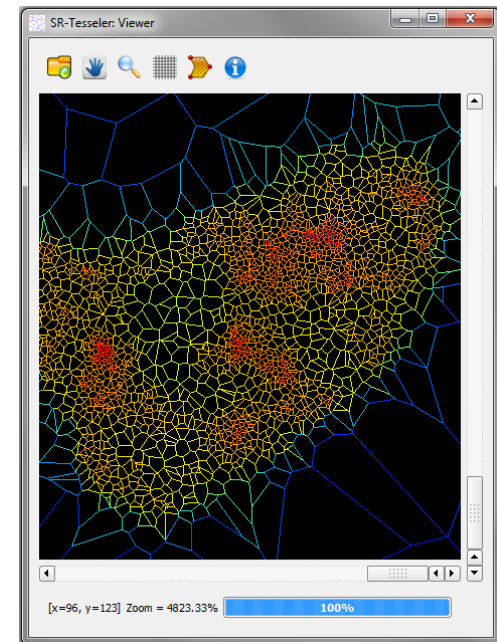


SR-Tesseler: Voronoï diagram (3/3)

- Once the Voronoï diagram created, the histogram of local densities is displayed
- Outline of the Voronoï polygons can be displayed (resp. hidden) by unchecking (resp. checking) the « Fill polygon » checkbox



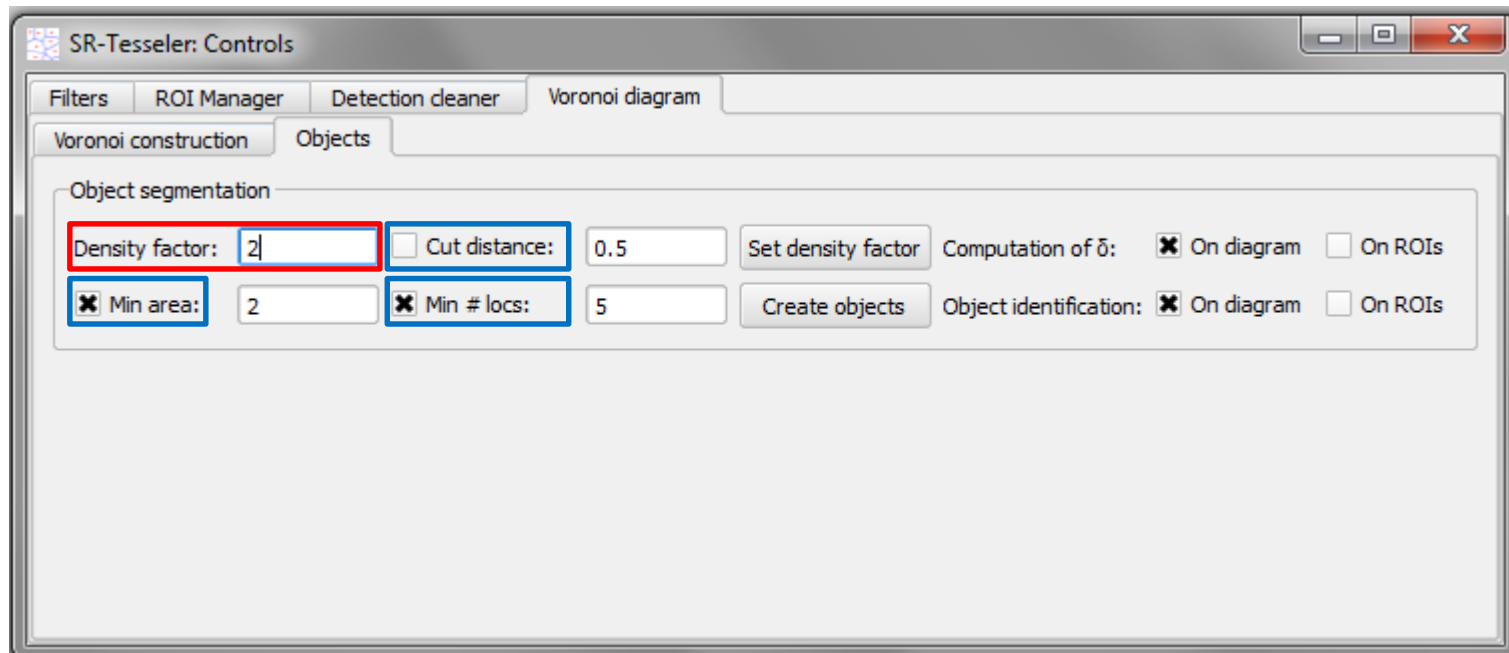
« Fill polygon » checked



« Fill polygon » unchecked

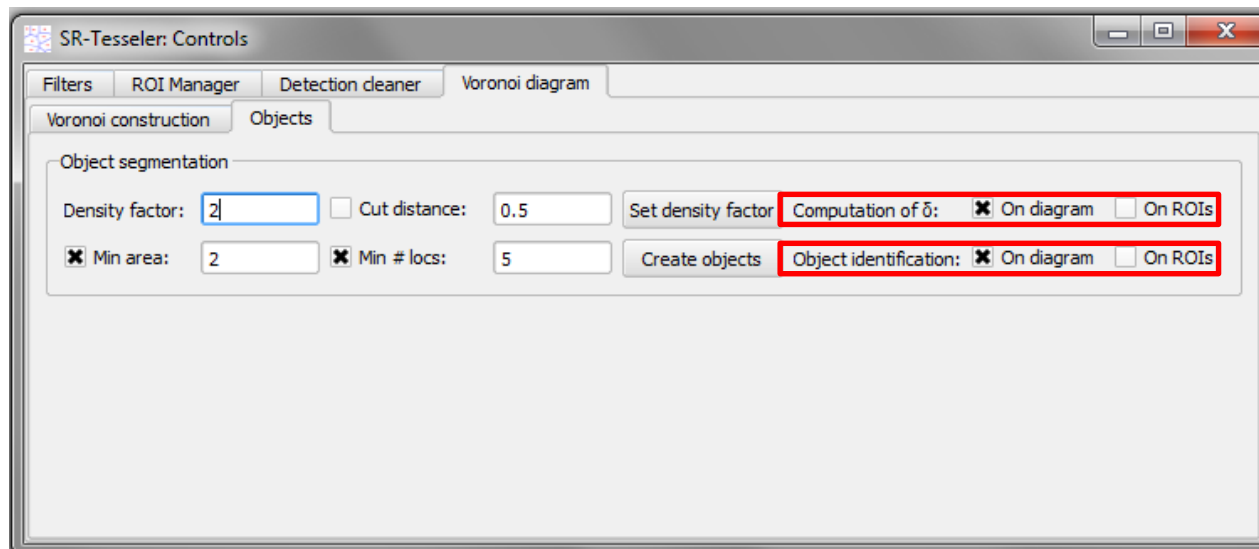
SR-Tesseler: Object creation (1/7)

- Adjust the density factor (of δ)
- Select and adjust the minimum area (in pixel²) and number of localizations of the objects, and a cut distance (in pixel) if needed



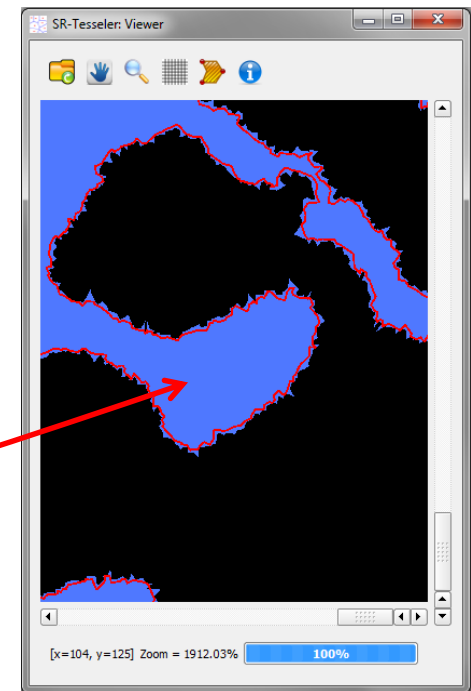
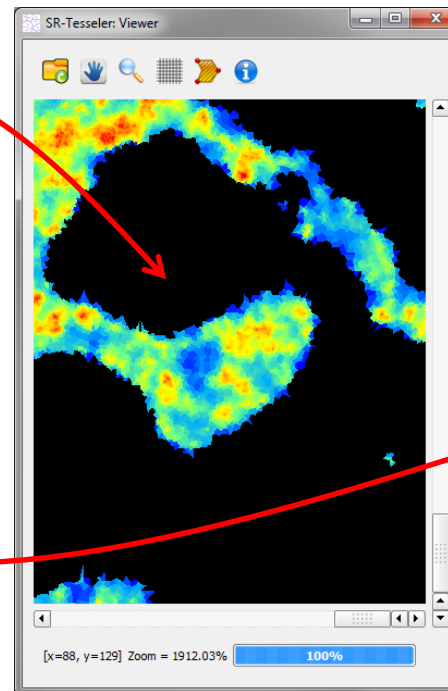
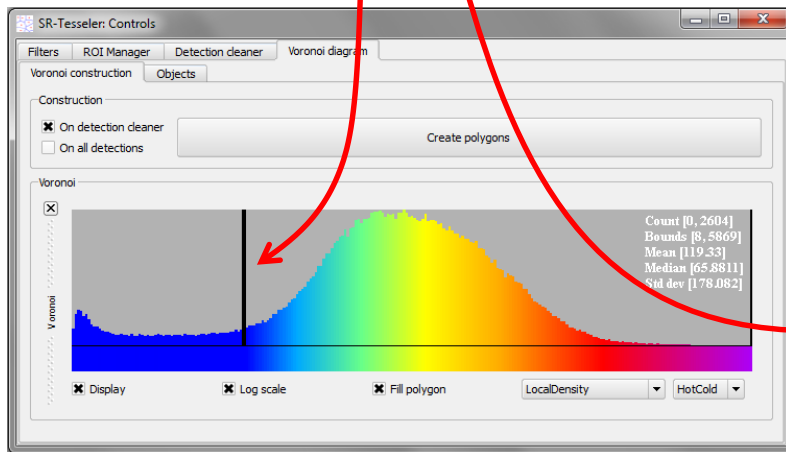
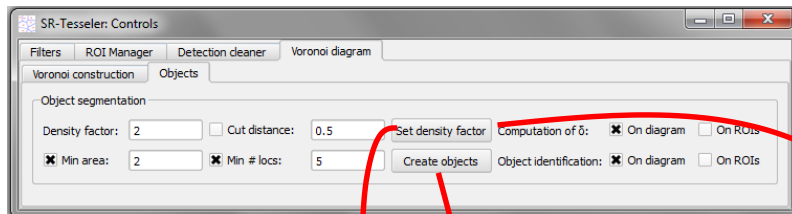
SR-Tesseler: Object creation (2/7)

- δ can be computed on the whole Voronoï diagram or on a subpart defined by the ROIs
- After setting the density factor, objects can be created on the whole Voronoï diagram or only on a subpart defined by the ROIs (example in slide 6/7)



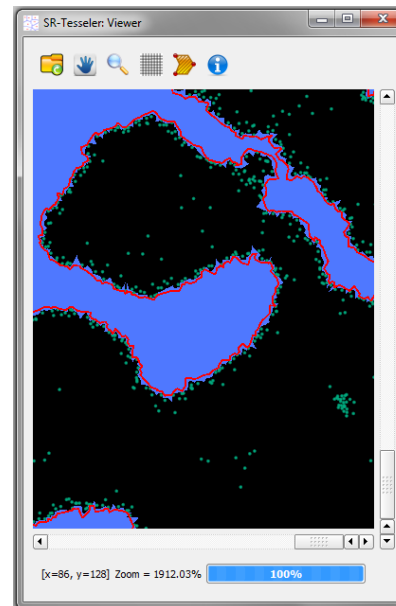
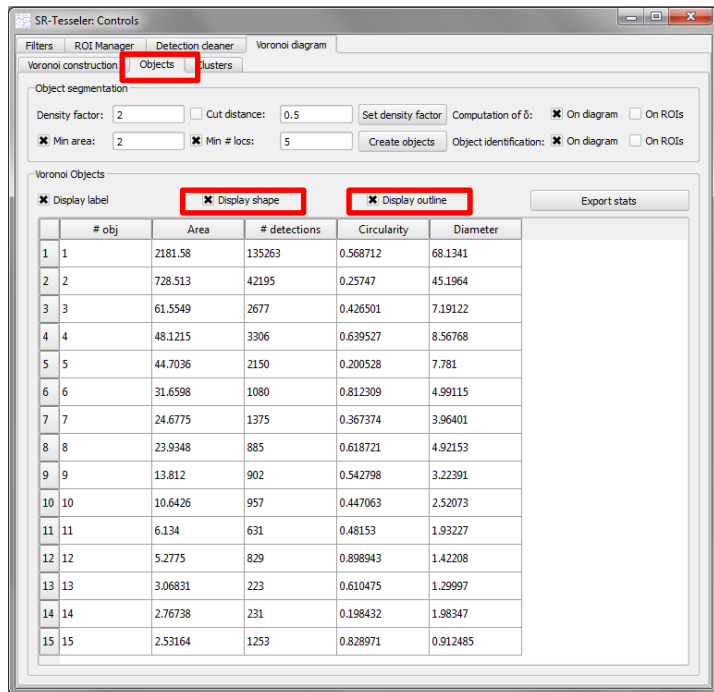
SR-Tesseler: Object creation (3/7)

- Click « Set density factor » then « Create objects » to compute the object

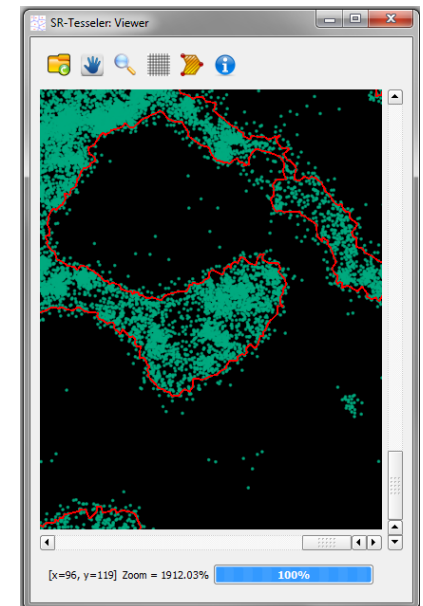


SR-Tesseler: Object creation (4/7)

- Object information are displayed in the « Objects » tab
- Object's shape and outline can be displayed by checking the « Display shape » and « Display outline » checkboxes respectively



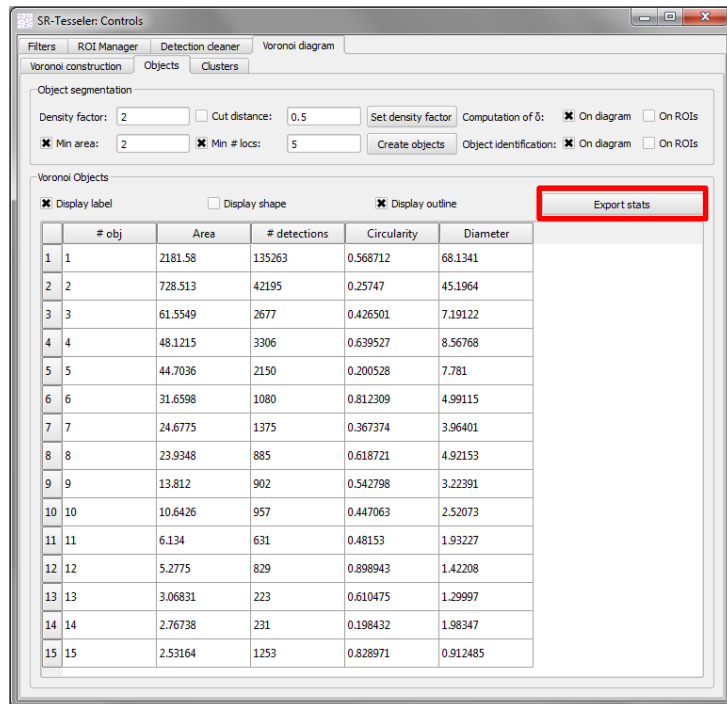
« Display shape » checked



« Display outline » checked

SR-Tesseler: Object creation (5/7)

- Object information can be exported as an Excel file in the data directory clicking the « Export stats » button

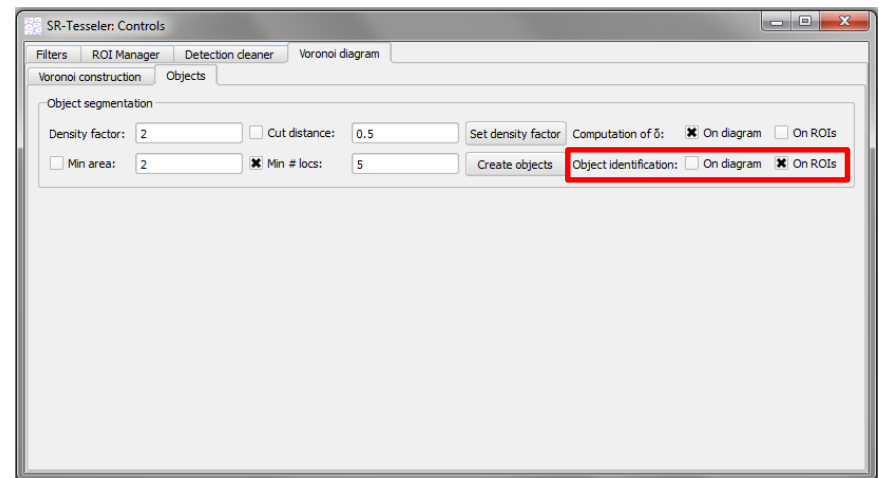
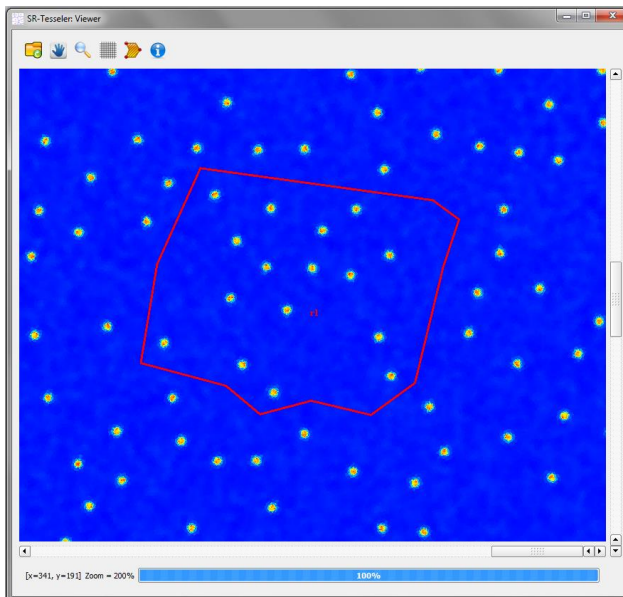


The screenshot shows the 'ObjectsStats.xls' Excel file. The table has 10 columns: '# object', '#roi', '#cluster', 'Area', '# detections', 'Local density', 'Major axis', 'Minor axis', 'Circularity', and 'Diameter'. The table contains 15 rows of data, corresponding to the data in the SR-Tesseler window.

# object	#roi	#cluster	Area	# detections	Local density	Major axis	Minor axis	Circularity	Diameter
1	-	-	2181.58	135263	62.0023	86.8662	49.4019	0.568712	68.1341
2	-	-	728.513	42195	57.9193	71.8847	18.5081	0.25747	45.1964
3	-	-	61.5549	2677	43.4896	10.0823	4.30012	0.426501	7.19122
4	-	-	48.1215	3306	68.7011	10.4514	6.68396	0.639527	8.56768
5	-	-	44.7036	2150	48.0946	12.9626	2.59937	0.200528	7.781
6	-	-	31.6598	1080	34.1126	5.50806	4.47424	0.812309	4.99115
7	-	-	24.6775	1375	55.7189	5.79799	2.13003	0.367374	3.96401
8	-	-	23.9348	885	36.9755	6.08076	3.76229	0.618721	4.92153
9	-	-	13.812	902	65.3055	4.1793	2.26852	0.542798	3.22391
10	-	-	10.6426	957	89.922	3.48393	1.55753	0.447063	2.52073
11	-	-	6.134	631	102.869	2.60848	1.25606	0.48153	1.93227
12	-	-	5.2775	829	157.082	1.49775	1.3464	0.898943	1.42208
13	-	-	3.06831	223	72.6785	1.61439	0.985547	0.610475	1.29997
14	-	-	2.76738	231	83.4725	3.3101	0.65683	0.198432	1.98347
15	-	-	2.53164	1253	494.935	0.997813	0.827157	0.828971	0.912485

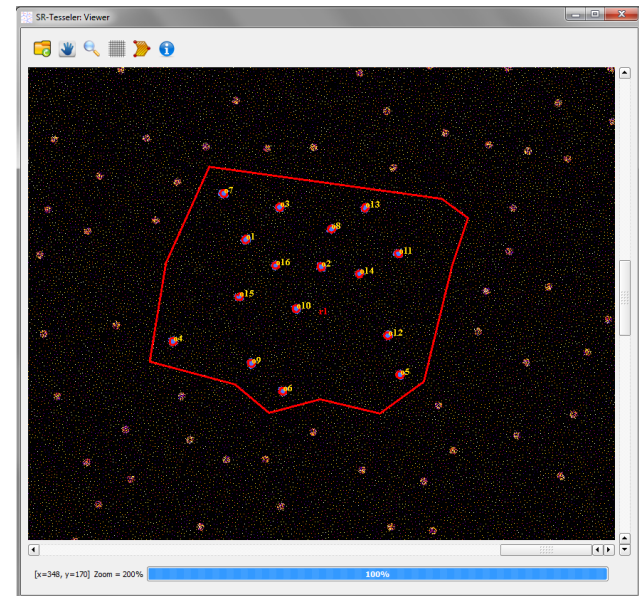
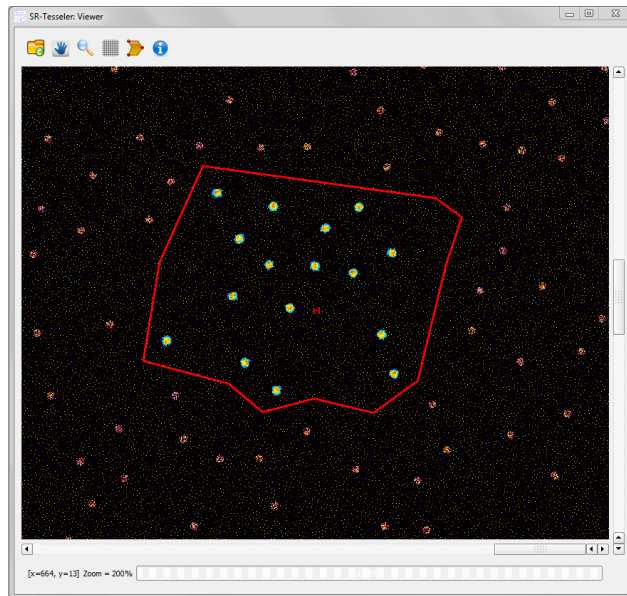
SR-Tesseler: Object creation (6/7)

- Example for object creation with ROIs
 - Create a ROI
 - Select « On ROIs » for the object creation
 - Click « Set density » then « Create objects »



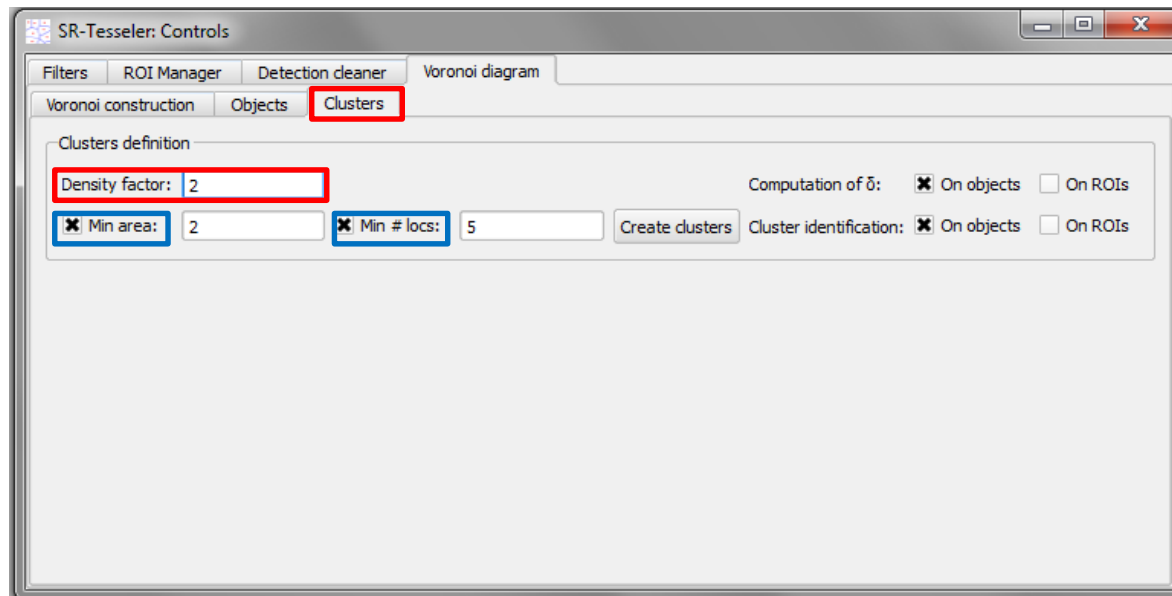
SR-Tesseler: Object creation (7/7)

- Example for object creation with ROIs
 - Create a ROI
 - Select « On ROIs » for the object creation
 - Click « Set density » then « Create objects »



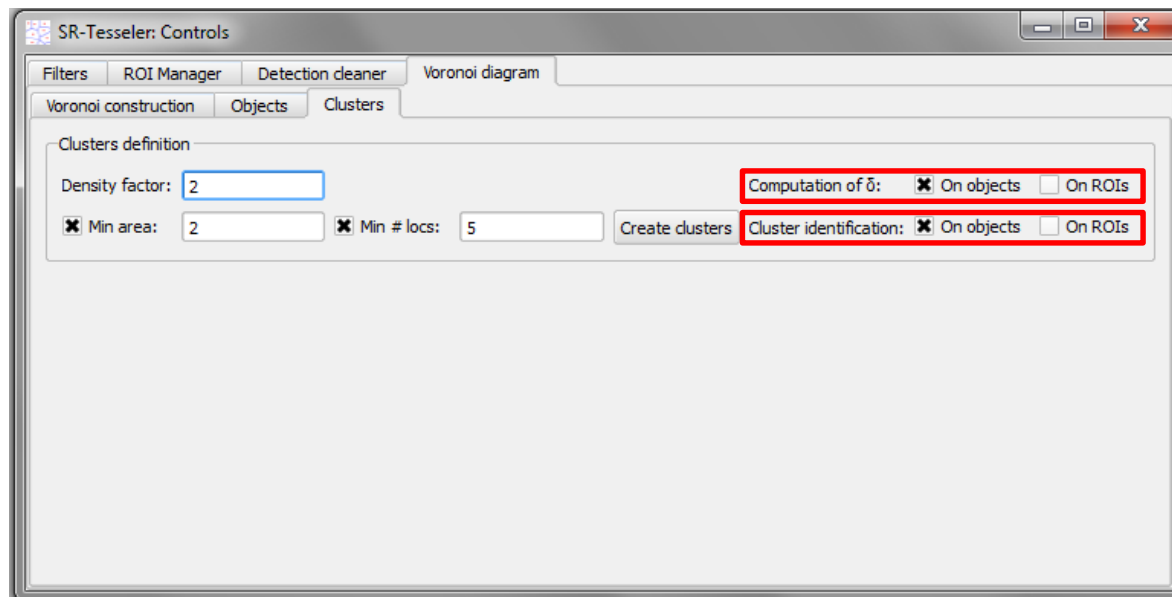
SR-Tesseler: Cluster creation (1/6)

- When one or more objects are identified, clusters can be computed from the « Clusters » tab
- Adjust the density factor (of δ)
- Select and adjust the minimum area (in pixel²) and number of detections per cluster



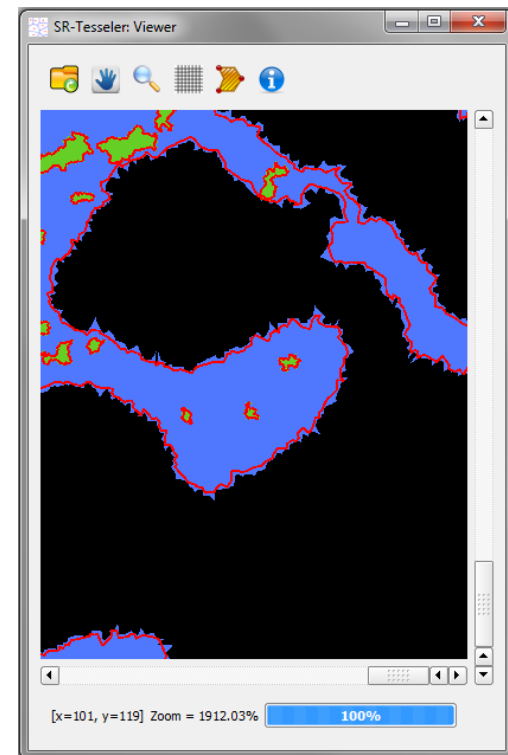
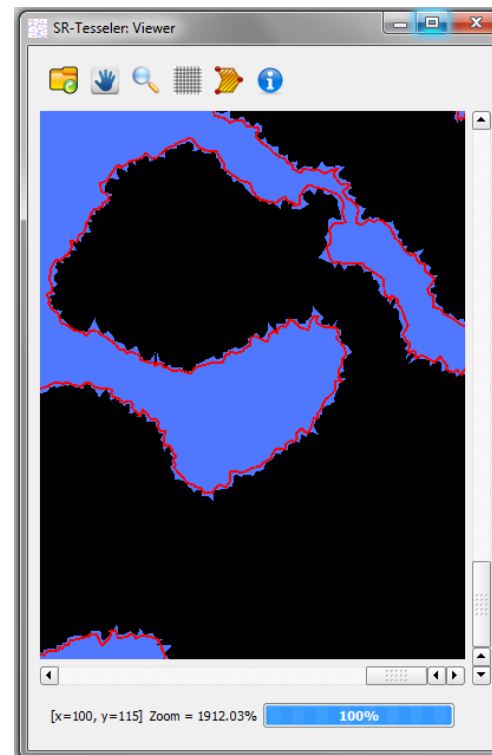
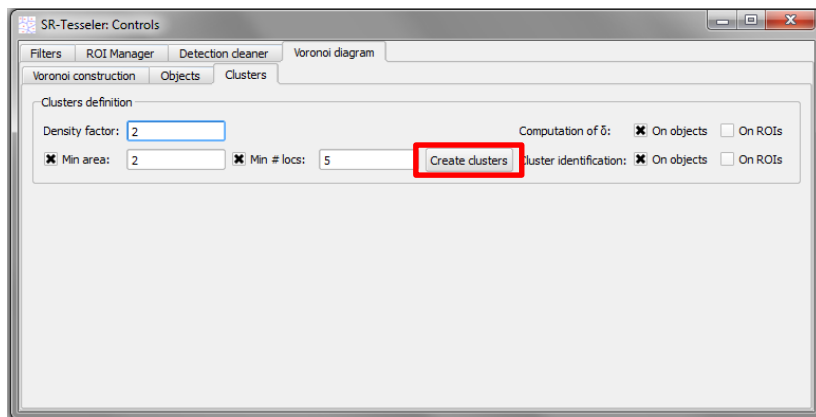
SR-Tesseler: Cluster creation (2/6)

- δ can be computed for each object or globally on all the objects inside the ROIs
- Clusters can be created for each object or only on the objects being inside the ROIs (example in slide 6/6)



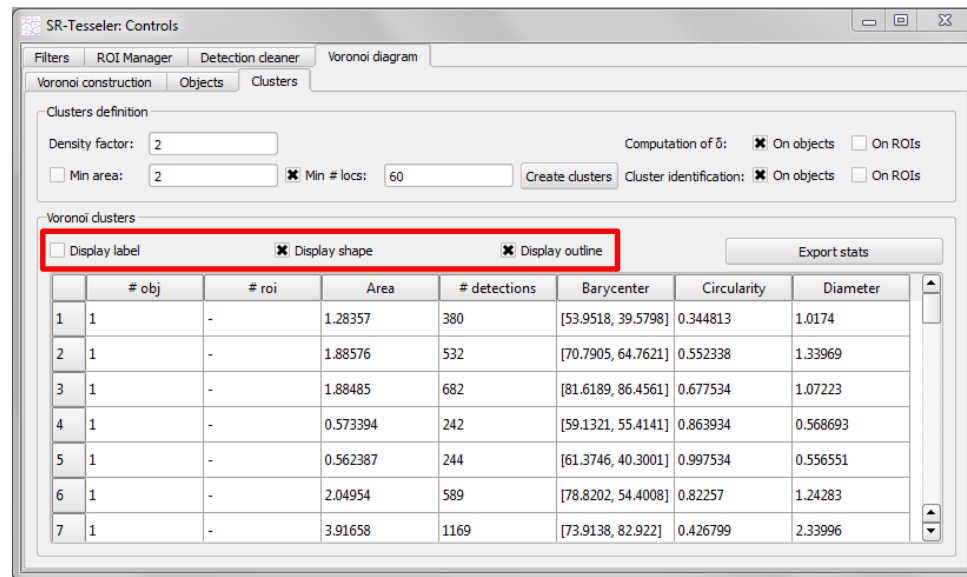
SR-Tesseler: Cluster creation (3/6)

- Click on « Create clusters » to compute clusters



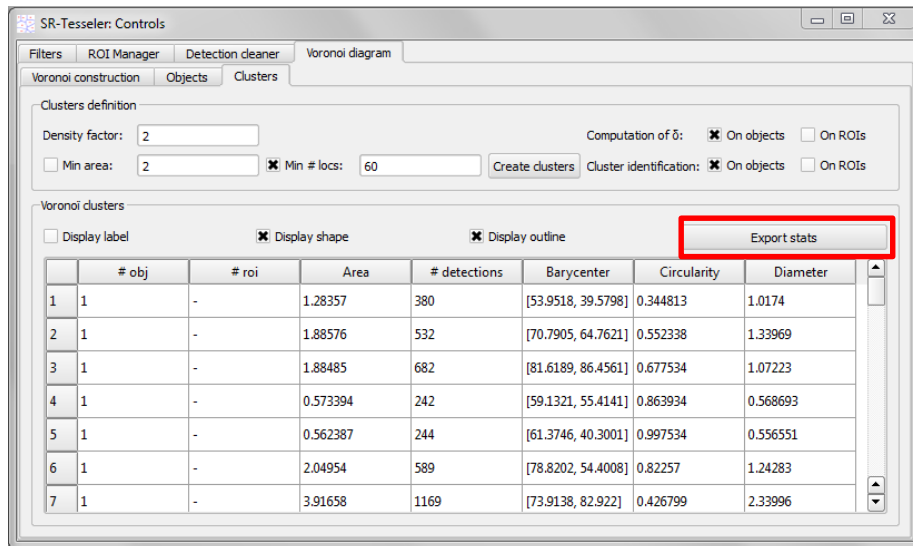
SR-Tesseler: Cluster creation (4/6)

- Clusters' shapes, outlines and labels can be displayed (resp. hidden) by checking (resp. unchecking) the corresponding checkboxes
- For each cluster, area (in pix^2), nb of detections, coordinates and morphological parameters (by principal component analysis) are computed and displayed



SR-Tesseler: Cluster creation (5/6)

- Cluster information can be exported as an Excel file in the data directory clicking the « Export stats » button

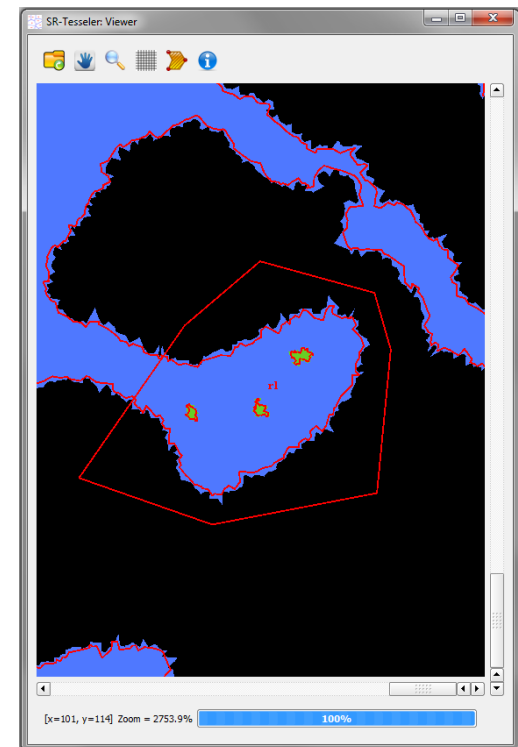
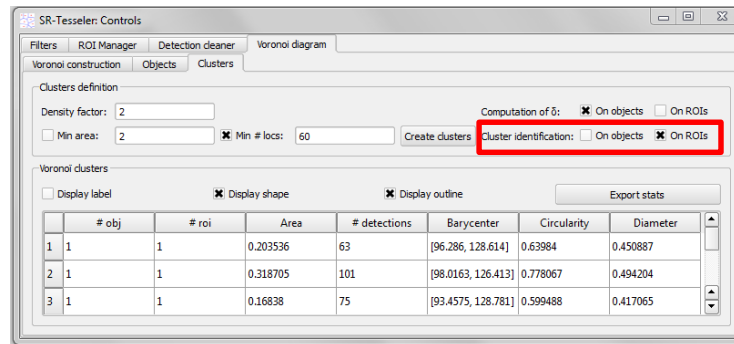
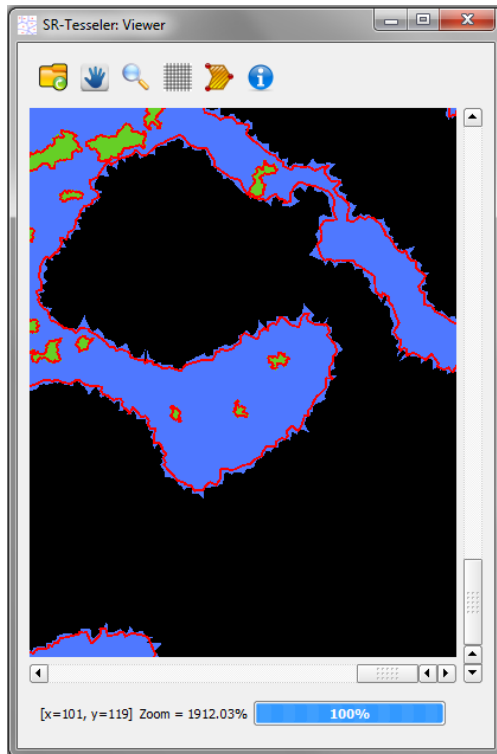


The screenshot shows the Excel file ObjectsAndClustersStats_factor_2_nbMinMolClusters_60.xls. The data is organized into columns A through J, corresponding to the fields in the Clusters table. The data is as follows:

	A	B	C	D	E	F	G	H	I	J
	# object	#roi	#cluster	Area	# detections	Local density	Major axis	Minor axis	Circularity	Diameter
1	1	-	-	2181.58	135263	62.0023	86.8662	49.4019	0.568712	68.1341
2	1	-	1	1.28357	380	296.05	1.51307	0.521727	0.344813	1.0174
3	1	-	2	1.88576	532	282.114	1.72602	0.953348	0.552338	1.33969
4	1	-	3	1.88485	682	361.833	1.27834	0.866115	0.677534	1.07223
5	1	-	4	0.573394	242	422.048	0.610208	0.527179	0.863934	0.568693
6	1	-	5	0.562387	244	433.865	0.557238	0.555864	0.997534	0.556551
7	1	-	6	2.04954	589	287.381	1.36383	1.12184	0.82257	1.24283
8	1	-	7	3.91658	1169	298.475	3.28001	1.3999	0.426799	2.33996
9	1	-	8	1.34164	410	305.596	1.38582	0.970528	0.70033	1.17817
10	1	-	9	2.0633	745	361.072	1.42861	0.739912	0.517926	1.08426
11	1	-	10	1.67222	499	298.405	1.45798	0.742008	0.50893	1.09999
12	1	-	11	0.293715	90	306.419	0.558548	0.498185	0.891929	0.528366
13	1	-	12	2.96395	936	315.795	2.05599	1.03397	0.502905	1.54498
14	1	-	13	0.747773	249	332.989	1.04732	0.68221	0.651384	0.864767
15	1	-	14	0.602412	163	270.579	0.871686	0.54118	0.620843	0.706433
16	1	-	15	1.81722	554	304.861	1.77135	0.952171	0.537539	1.36176
17	1	-	16	0.834556	248	297.164	0.804241	0.728233	0.90549	0.766237
18	1	-	17	5.21032	1527	293.072	3.11193	0.973437	0.312808	2.04269
19	1	-	18	1.56209	545	348.891	1.4192	0.79218	0.558189	1.10569
20	1	-	19	0.927774	344	370.78	0.915506	0.555074	0.606303	0.73529

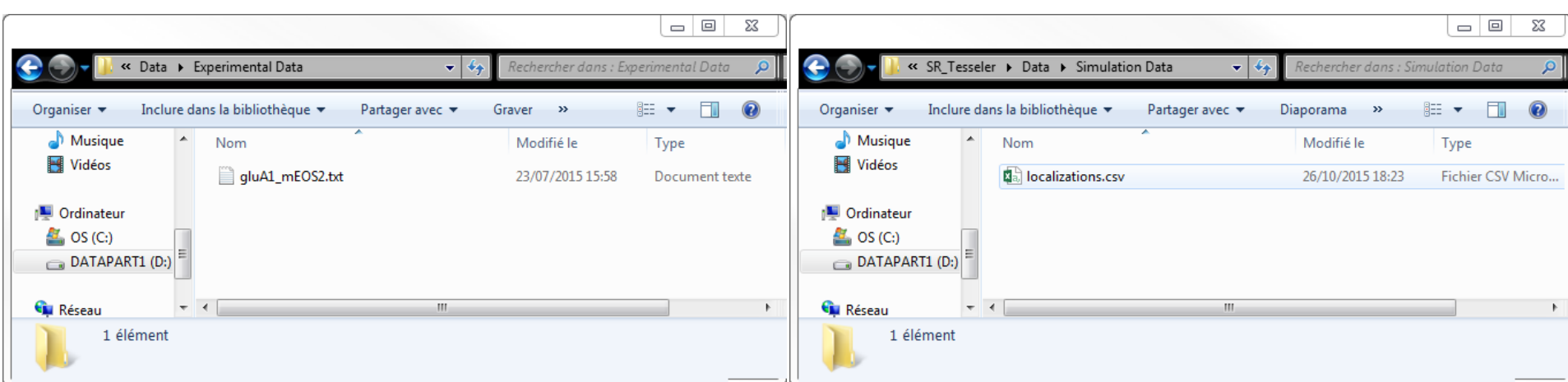
SR-Tesseler: Cluster creation (6/6)

- Example of clusters creation with “On ROIs” selected



Data format (1/3)

- In the data directory, one file describing the localizations is needed.
- Two formats are possible
 - An ASCII txt file
 - A CSV file

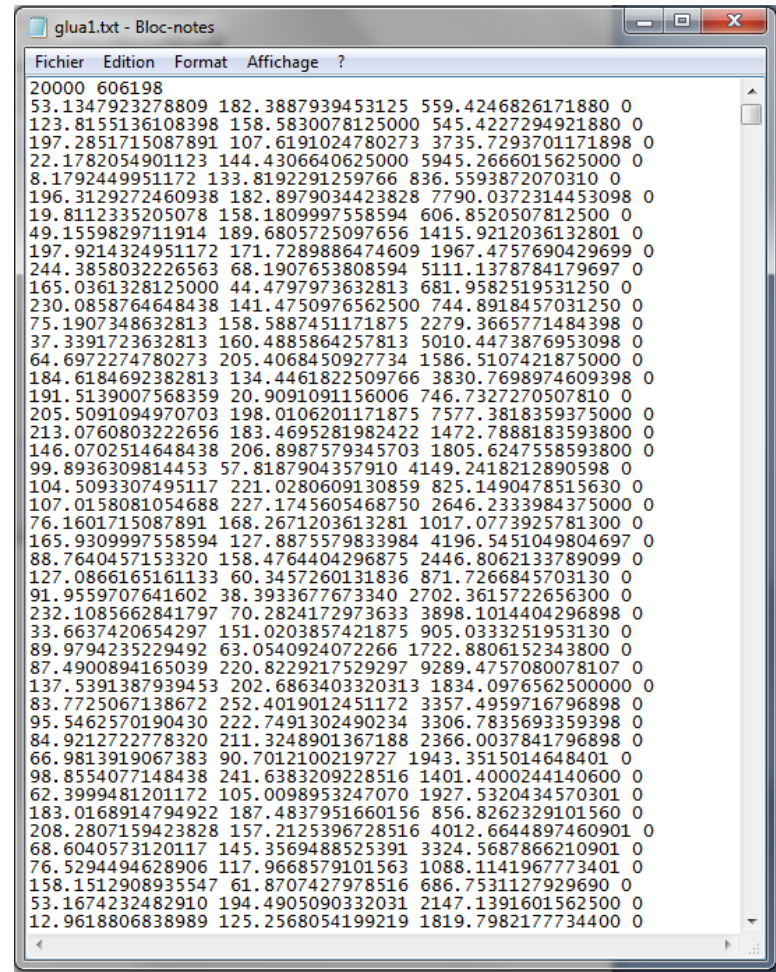


Data format (2/3)

- Detection ASCII file format

Header: [Nb planes, Nb detections]

List of detections
[x y intensity frame sigma]
sigma is optional



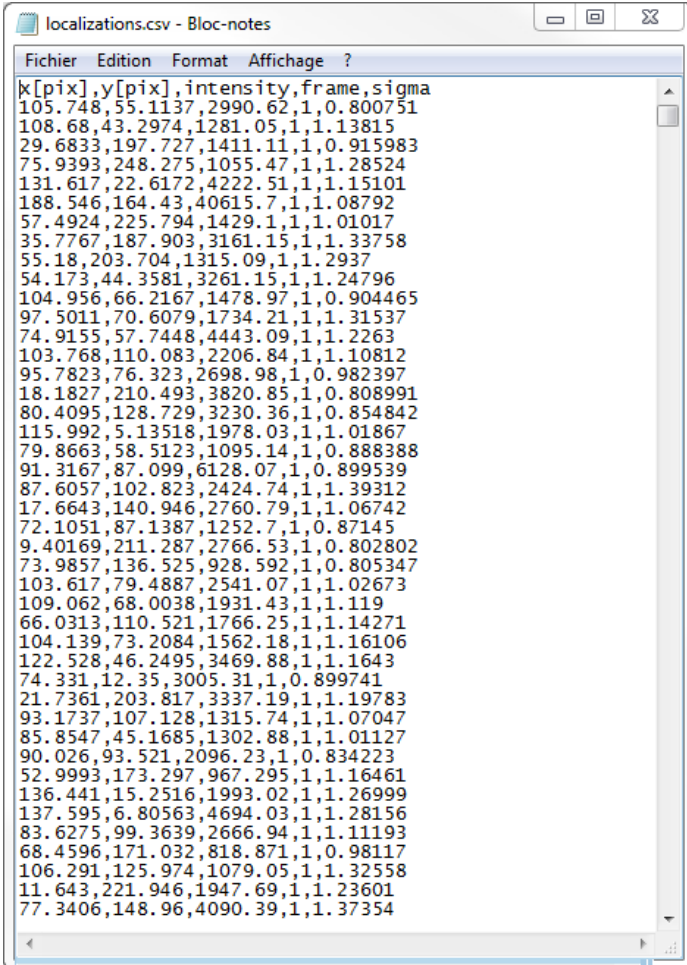
```
glua1.txt - Bloc-notes
Fichier  Edition  Format  Affichage  ?
20000 606198
53.1347923278809 182.3887939453125 559.4246826171880 0
123.8155136108398 158.5830078125000 545.4227294921880 0
197.2851715087891 107.6191024780273 3735.7293701171898 0
22.1782054901123 144.4306640625000 5945.2666015625000 0
8.1792449951172 133.8192291259766 836.5593872070310 0
196.3129272460938 182.8979034423828 7790.0372314453098 0
19.8112335205078 158.1809997558594 606.8520507812500 0
49.1559829711914 189.6805725097656 1415.9212036132801 0
197.9214324951172 171.7289886474609 1967.4757690429699 0
244.3858032226563 68.1907653808594 5111.1378784179697 0
165.0361328125000 44.4797973632813 681.9582519531250 0
230.0858764648438 141.4750976562500 744.8918457031250 0
75.1907348632813 158.5887451171875 2279.3665771484398 0
37.3391723632813 160.4885864257813 5010.4473876953098 0
64.6972274780273 205.4068450927734 1586.5107421875000 0
184.6184692382813 134.4461822509766 3830.7698974609398 0
191.5139007568359 20.9091091156006 746.7327270507810 0
205.5091094970703 198.0106201171875 7577.3818359375000 0
213.0760803222656 183.4695281982422 1472.7888183593800 0
146.0702514648438 206.8987579345703 1805.6247558593800 0
99.8936309814453 57.8187904357910 4149.2418212890598 0
104.5093307495117 221.0280609130859 825.1490478515630 0
107.0158081054688 227.1745605468750 2646.2339843750000 0
76.1601715087891 168.2671203613281 1017.0773925781300 0
165.9309997558594 127.8875579833984 4196.5451049804697 0
88.7640457153320 158.4764404296875 2446.8062133789099 0
127.0866165161133 60.3457260131836 871.7266845703130 0
91.9559707641602 38.3933677673340 2702.3615722656300 0
232.1085662841797 70.2824172973633 3898.1014404296898 0
33.6637420654297 151.0203857421875 905.0333251953130 0
89.9794235229492 63.0540924072266 1722.8806152343800 0
87.4900894165039 220.8229217529297 9289.4757080078107 0
137.5391387939453 202.6863403320313 1834.0976562500000 0
83.7725067138672 252.4019012451172 3357.4959716796898 0
95.5462570190430 222.7491302490234 3306.7835693359398 0
84.9212722778320 211.3248901367188 2366.0037841796898 0
66.9813919067383 90.7012100219727 1943.3515014648401 0
98.8554077148438 241.6383209228516 1401.4000244140600 0
62.3999481201172 105.0098953247070 1927.5320434570301 0
183.0168914794922 187.4837951660156 856.8262329101560 0
208.2807159423828 157.2125396728516 4012.6644897460901 0
68.6040573120117 145.3569488525391 3324.5687866210901 0
76.5294494628906 117.9668579101563 1088.1141967773401 0
158.1512908935547 61.8707427978516 686.7531127929690 0
53.1674232482910 194.4905090332031 2147.1391601562500 0
12.9618806838989 125.2568054199219 1819.7982177734400 0
```

Data format (3/3)

- CSV file format

Header line
sigma is optional

List of detections
[x,y,intensity,frame,sigma]
sigma is optional



```
localizations.csv - Bloc-notes
Fichier  Edition  Format  Affichage  ?
x[pix],y[pix],intensity,frame,sigma
105.748,55.1137,2990.62,1,0.800751
108.68,43.2974,1281.05,1,1.13815
29.6833,197.727,1411.11,1,0.915983
75.9393,248.275,1055.47,1,1.28524
131.617,22.6172,4222.51,1,1.15101
188.546,164.43,40615.7,1,1.08792
57.4924,225.794,1429.1,1,1.01017
35.7767,187.903,3161.15,1,1.33758
55.18,203.704,1315.09,1,1.2937
54.173,44.3581,3261.15,1,1.24796
104.956,66.2167,1478.97,1,0.904465
97.5011,70.6079,1734.21,1,1.31537
74.9155,57.7448,4443.09,1,1.2263
103.768,110.083,2206.84,1,1.10812
95.7823,76.323,2698.98,1,0.982397
18.1827,210.493,3820.85,1,0.808991
80.4095,128.729,3230.36,1,0.854842
115.992,5.13518,1978.03,1,1.01867
79.8663,58.5123,1095.14,1,0.888388
91.3167,87.099,6128.07,1,0.899539
87.6057,102.823,2424.74,1,1.39312
17.6643,140.946,2760.79,1,1.06742
72.1051,87.1387,1252.7,1,0.87145
9.40169,211.287,2766.53,1,0.802802
73.9857,136.525,928.592,1,0.805347
103.617,79.4887,2541.07,1,1.02673
109.062,68.0038,1931.43,1,1.119
66.0313,110.521,1766.25,1,1.14271
104.139,73.2084,1562.18,1,1.16106
122.528,46.2495,3469.88,1,1.1643
74.331,12.35,3005.31,1,0.899741
21.7361,203.817,3337.19,1,1.19783
93.1737,107.128,1315.74,1,1.07047
85.8547,45.1685,1302.88,1,1.01127
90.026,93.521,2096.23,1,0.834223
52.9993,173.297,967.295,1,1.16461
136.441,15.2516,1993.02,1,1.26999
137.595,6.80563,4694.03,1,1.28156
83.6275,99.3639,2666.94,1,1.11193
68.4596,171.032,818.871,1,0.98117
106.291,125.974,1079.05,1,1.32558
11.643,221.946,1947.69,1,1.23601
77.3406,148.96,4090.39,1,1.37354
```

Known limitations (1/2)

- For the 32 bits windows environment
 - Internal memory used by SR-Tesseler is limited
 - Maximum number of localizations for the Voronoi diagram creation is $\approx 1,100,000$ localizations
- There should be no such limitation with the 64 bits windows environment installer

Known limitations (2/2)

- If the internal memory of the graphic card is limited (for a laptop for instance), the Voronoï diagram is created but may not be displayed. All quantifications will work except for the diagram displaying.