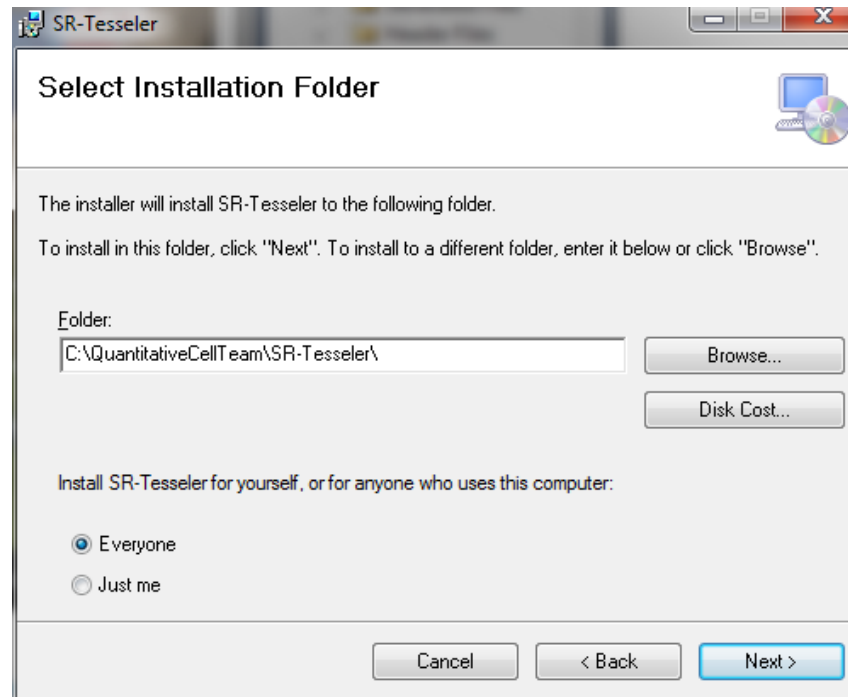# SR-TESSELER
Installation guide & Manual

By F. Levet & JB. Sibarita
Team Quantitative Imaging of the Cell
Interdisciplinary Institute for Neuroscience
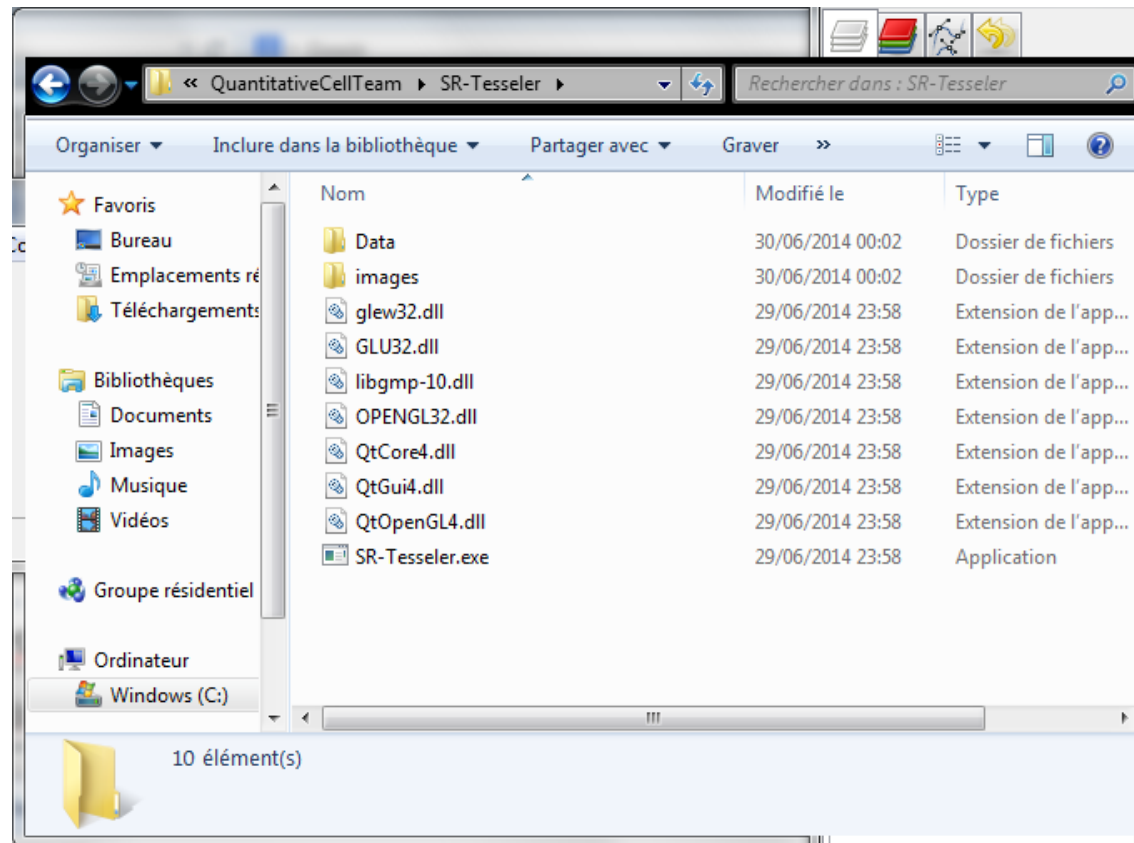CNRS UMR5297, Univ. Bordeaux

Installation (1/3)

- Need a PC with OpenGL installed

- Launch « Setup.msi »

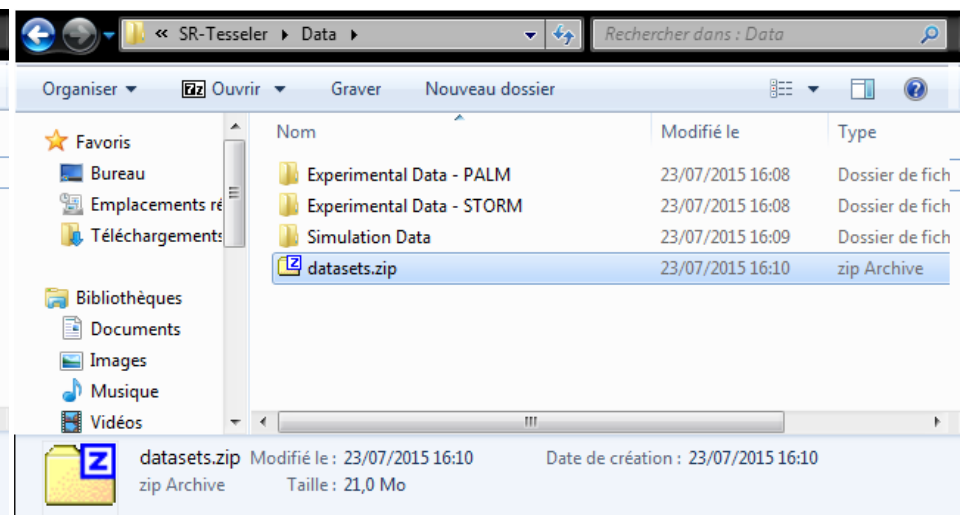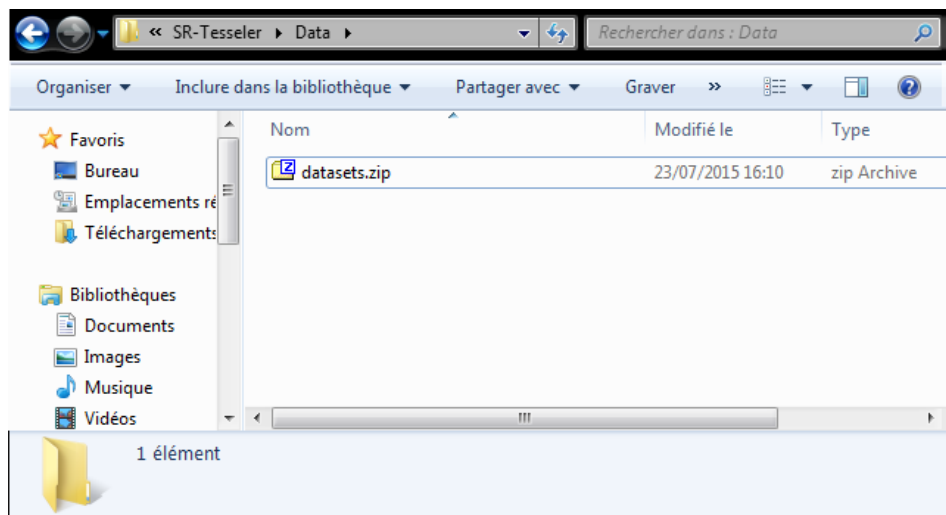- Select installation directory

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)
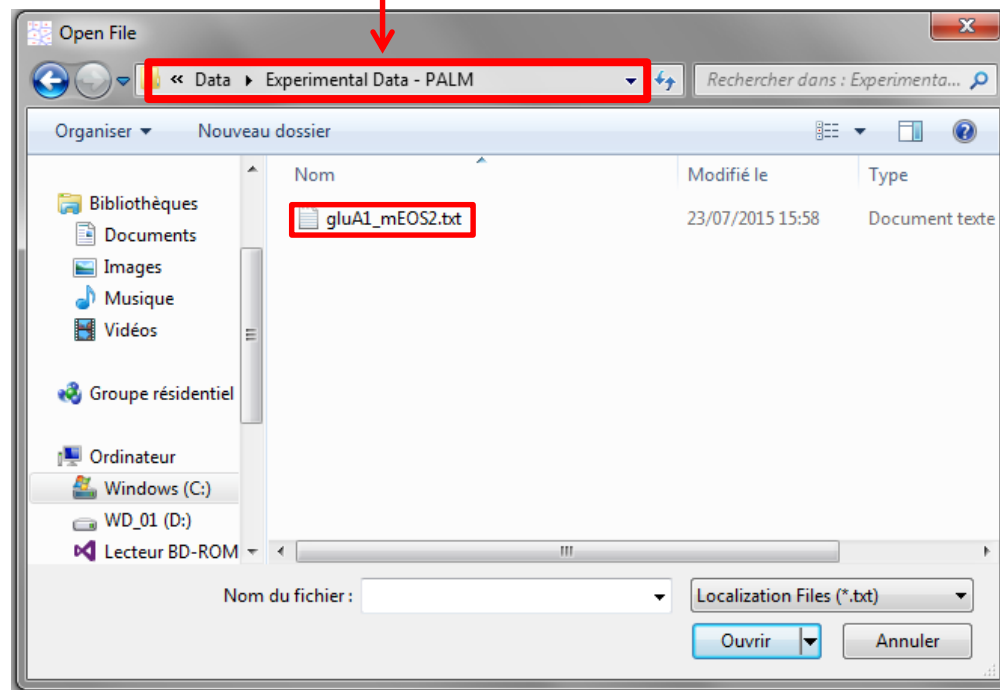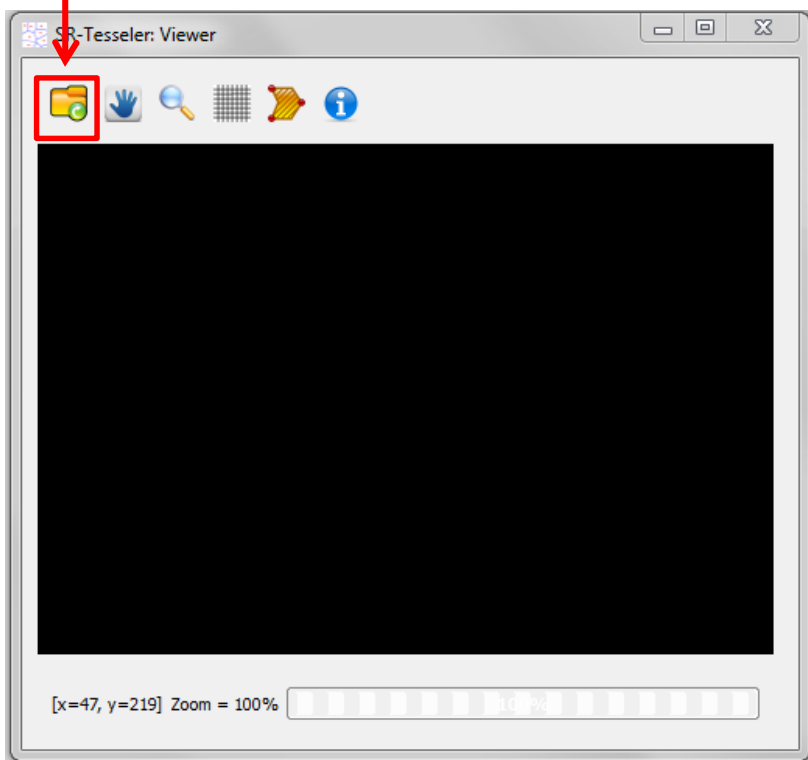
SR-Tesseler Quick start (1/2)

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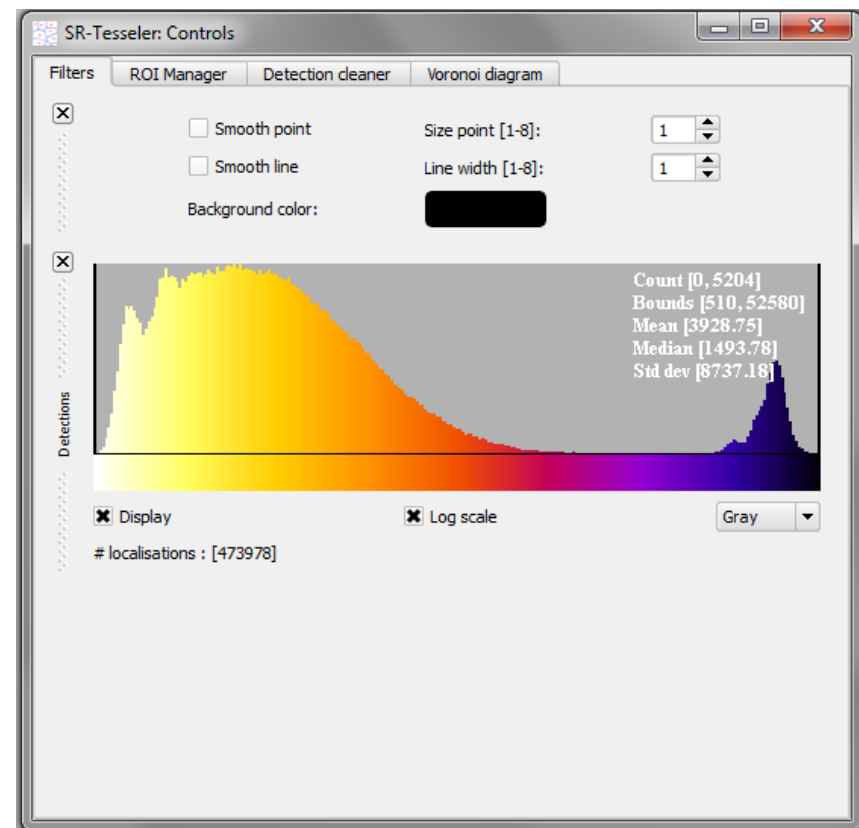 - a Console (for application messages)
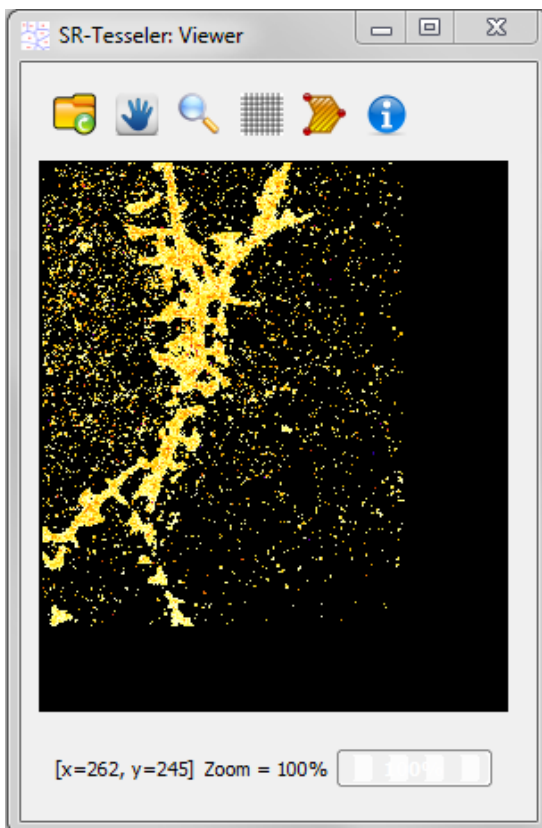 - A Viewer
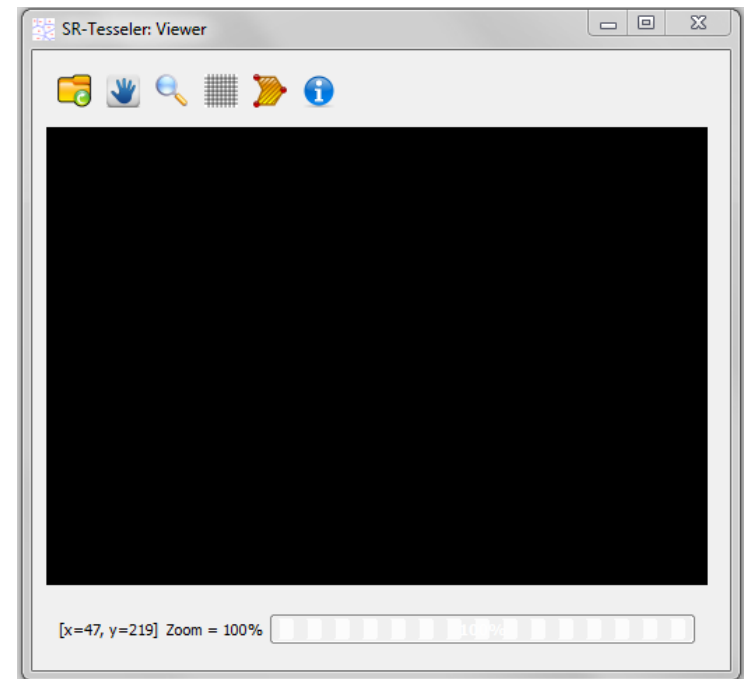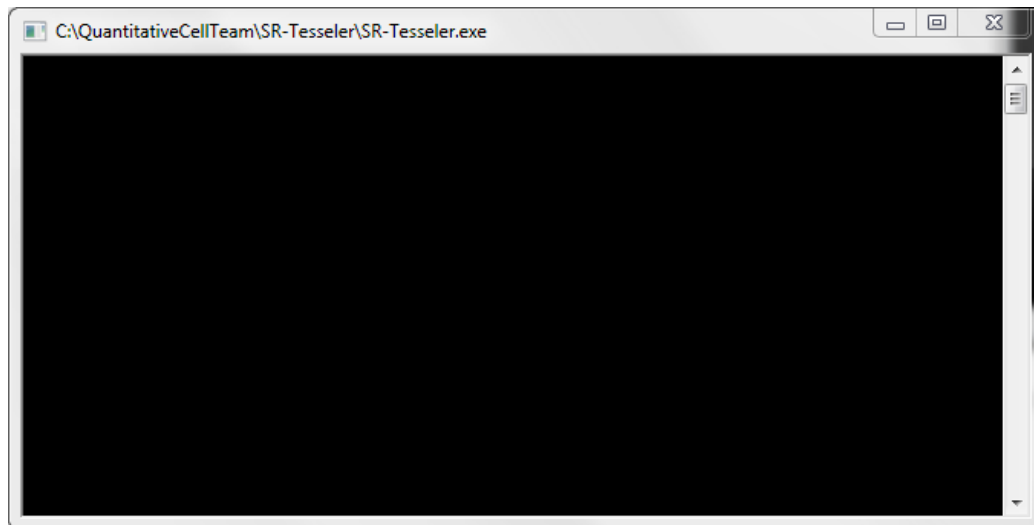
SR-Tesseler Quick start (2/2)

- To open single-molecule data:
  - Click on the open icon
  - Select localization file (e.g. « \Experimental Data - PALM\gluA1_mEOS2.txt »)
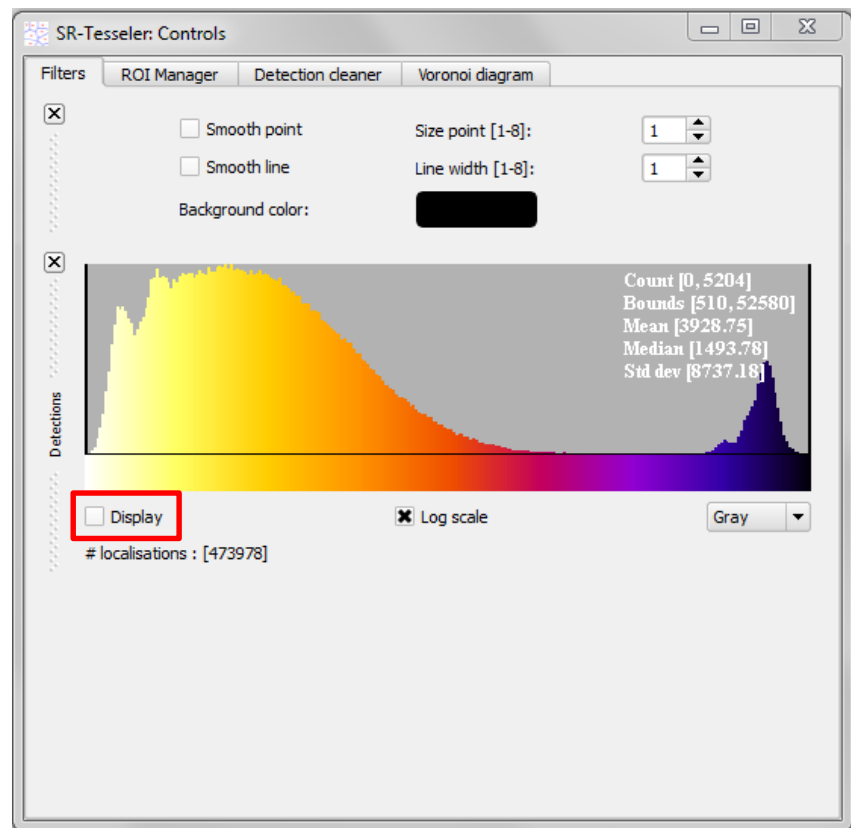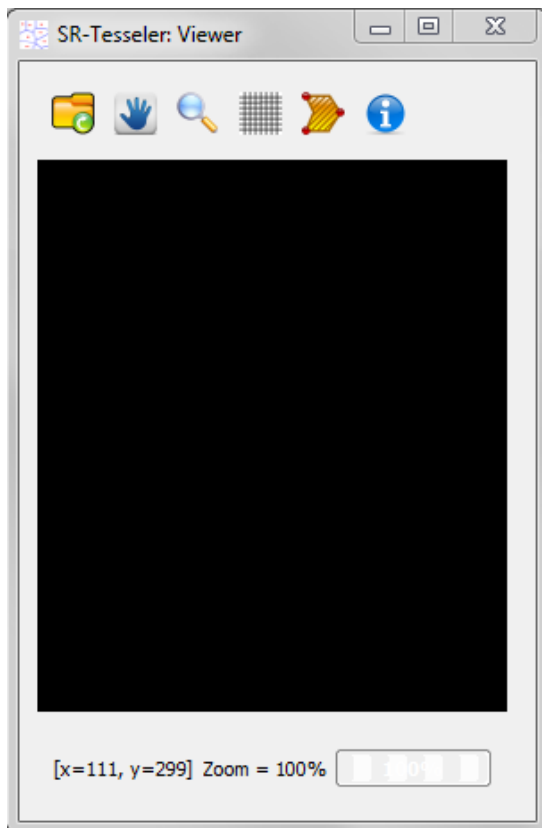
SR-Tesseler GUI (1/7)

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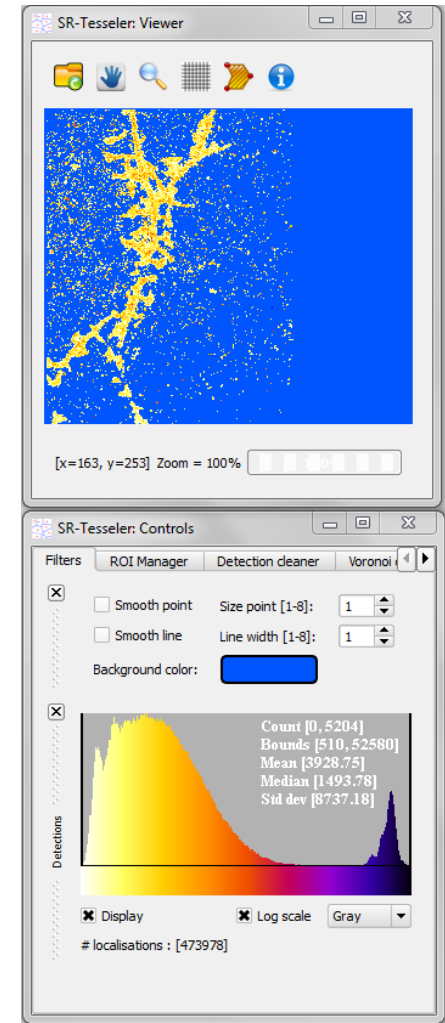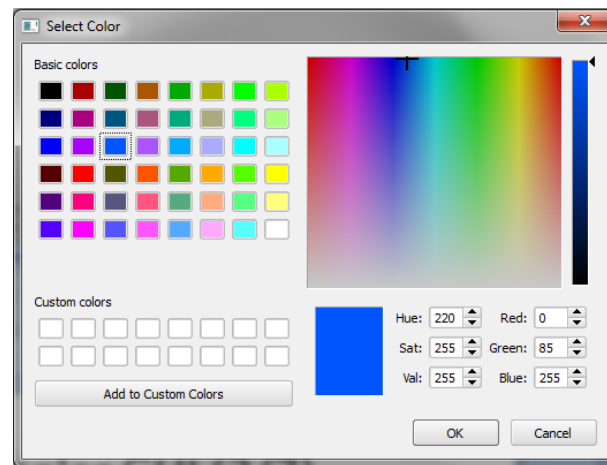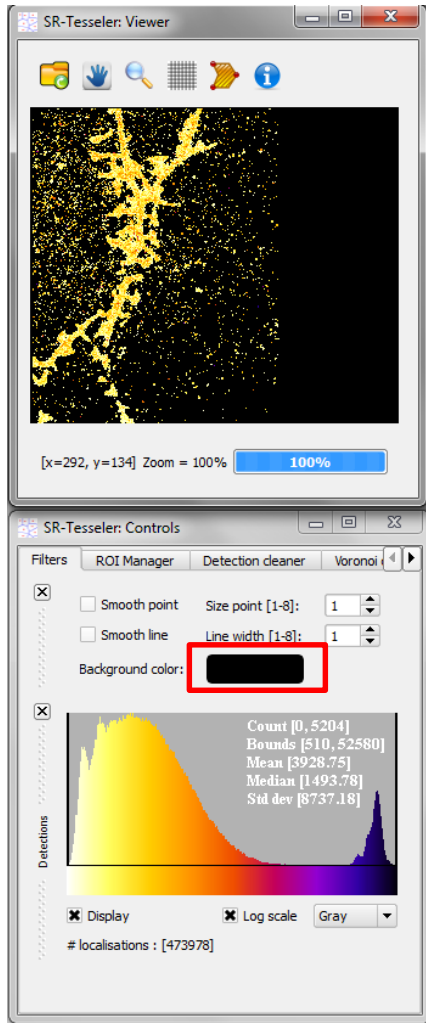 - An control window with histogram and options is displayed

SR-Tesseler GUI (2/7)

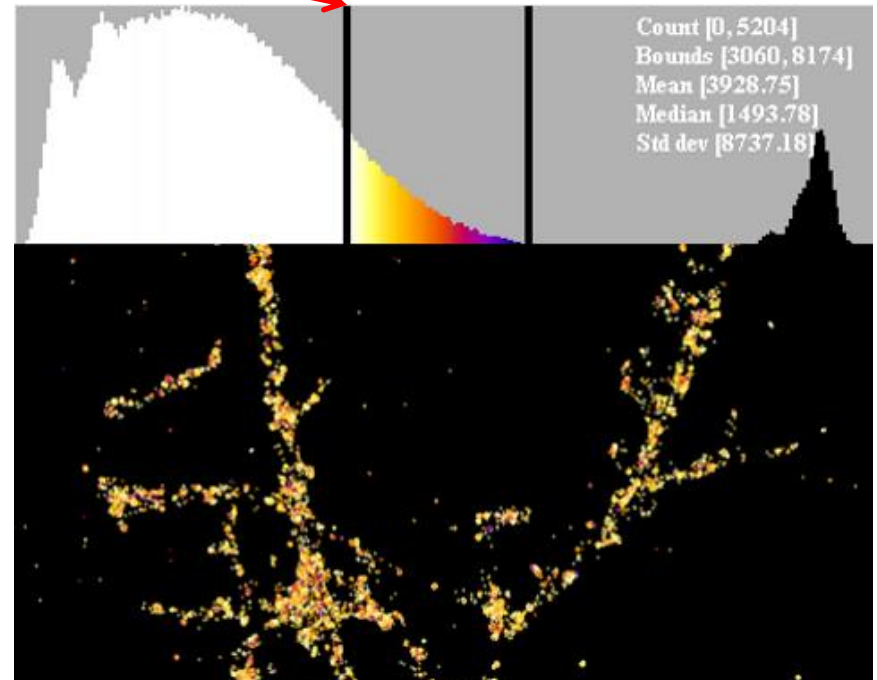- Single-molecule detections can be displayed by checking the « Display » check-boxes
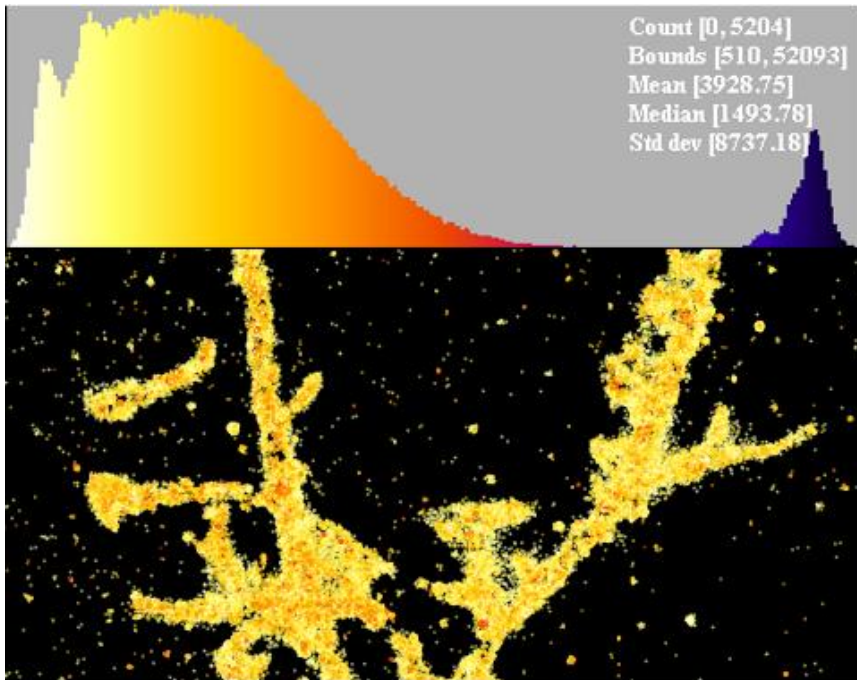
SR-Tesseler GUI (3/7)

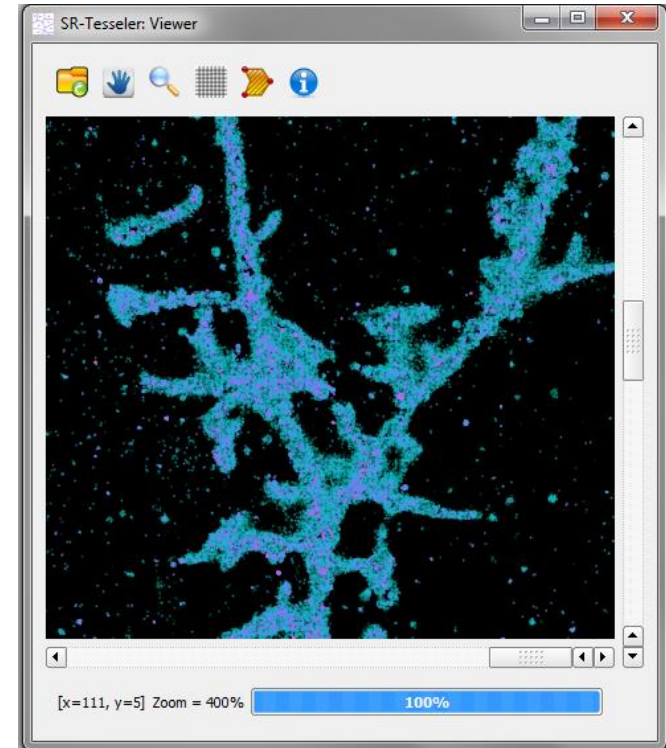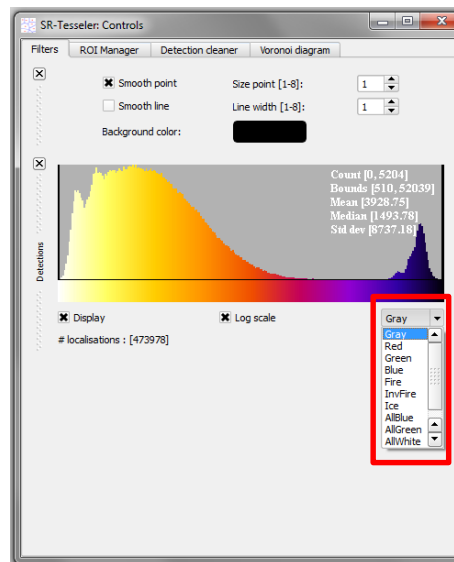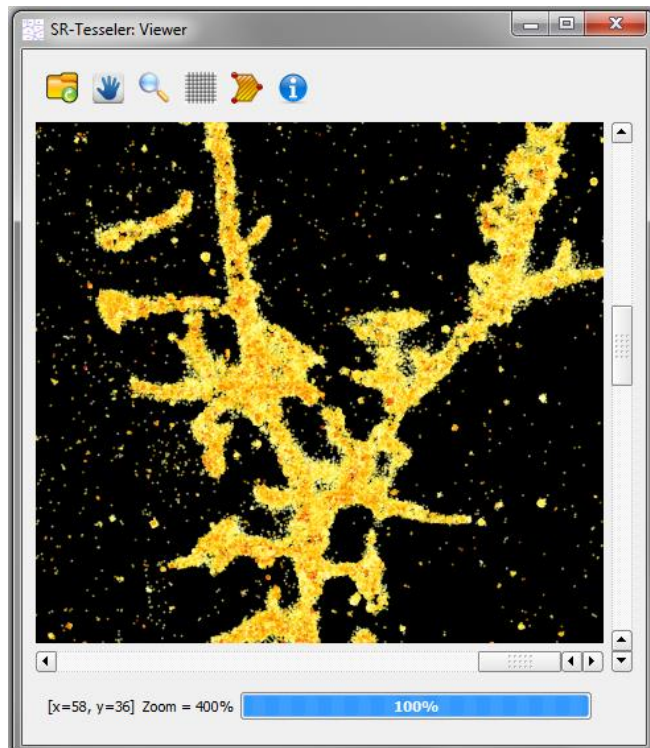- Click on the background color to change it

SR-Tesseler GUI (4/7)

- Intensity scaling is performed by clicking on the histogram: left (resp. right) selects for min (resp. max)
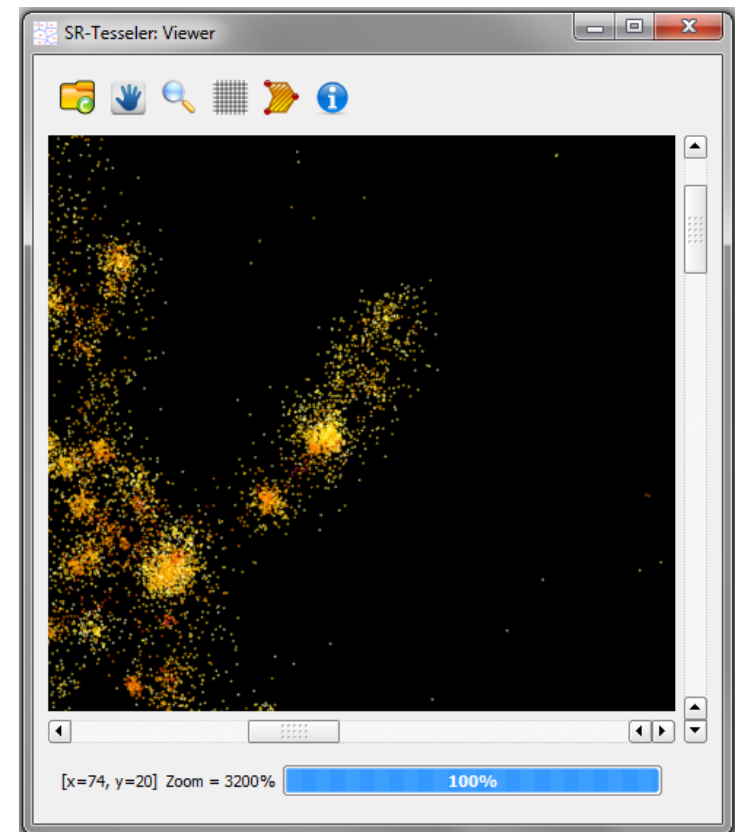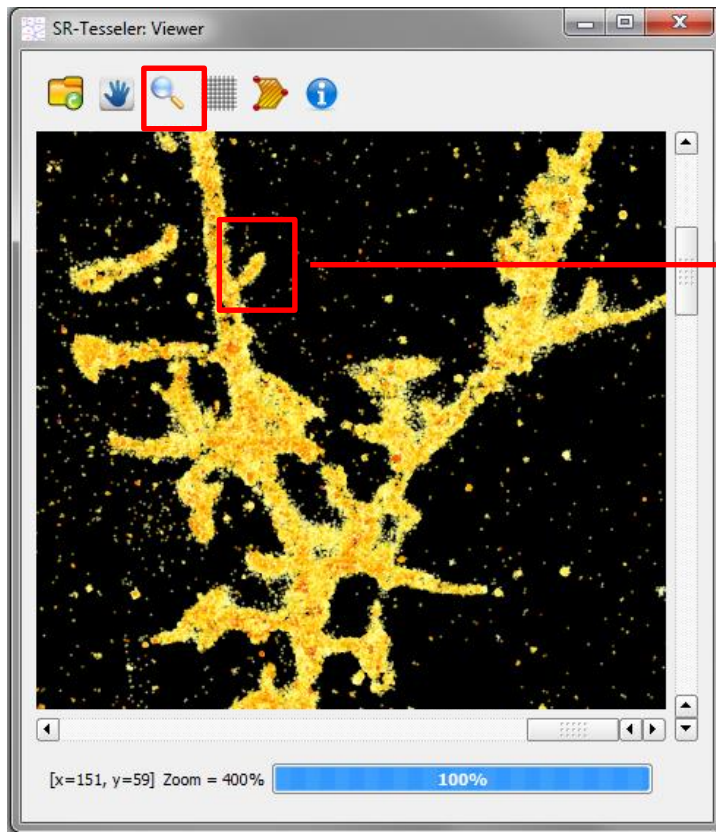
SR-Tesseler GUI (5/7)
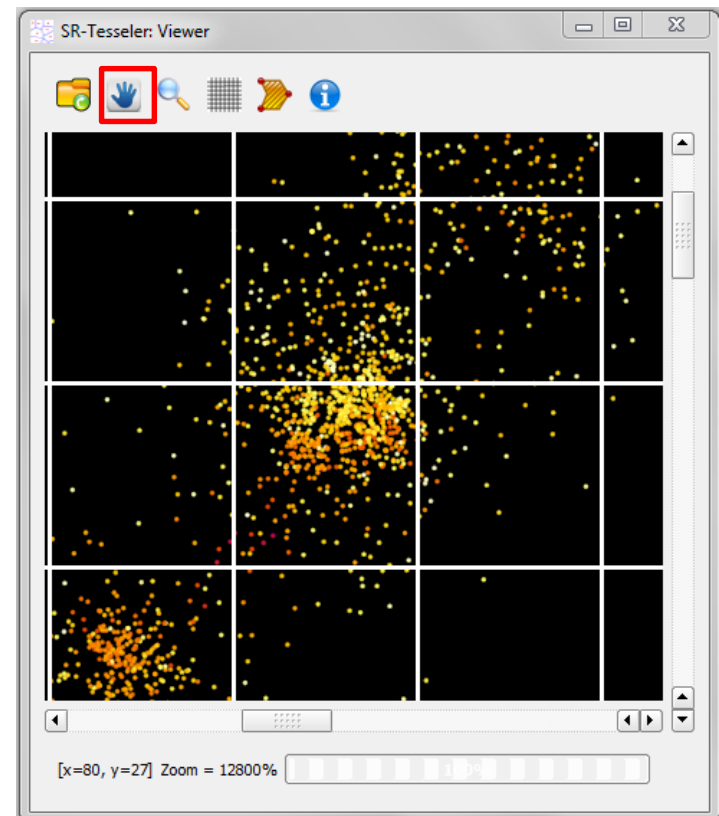
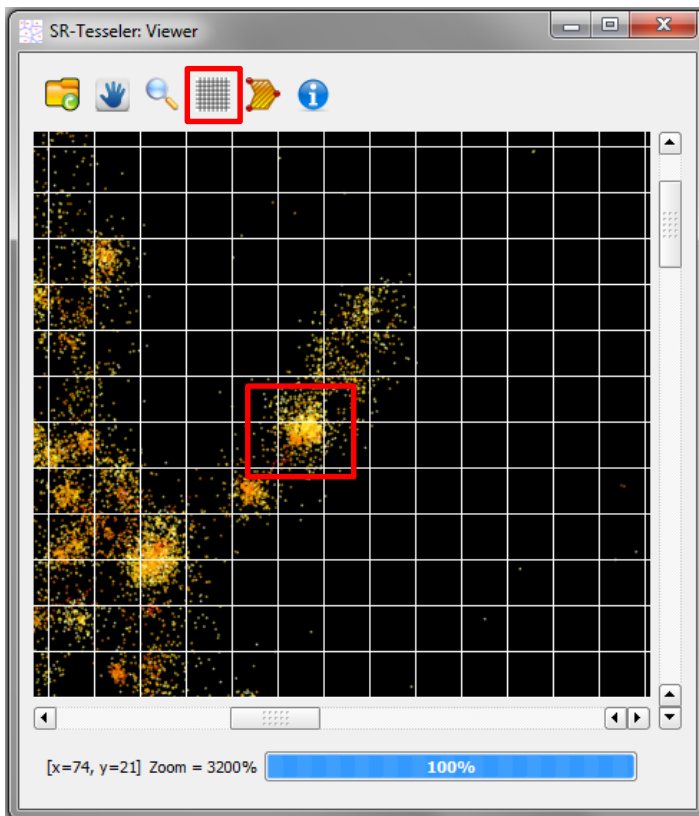- Change the LUT by clicking on the combo box.

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

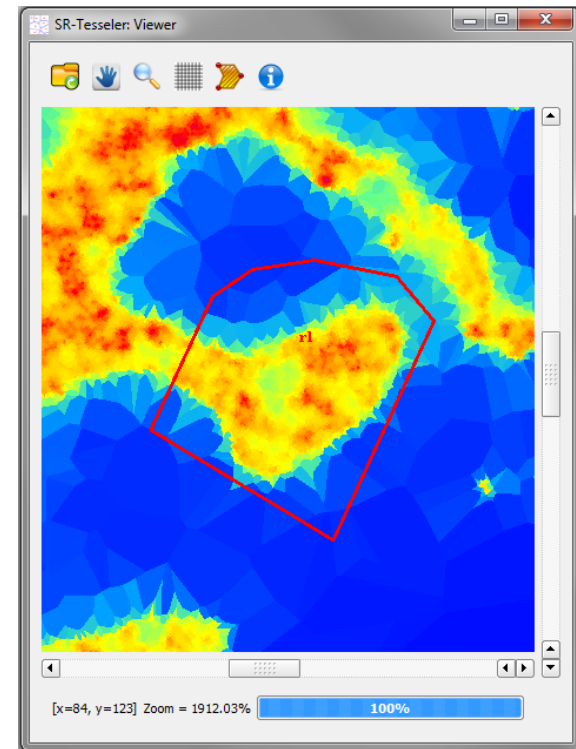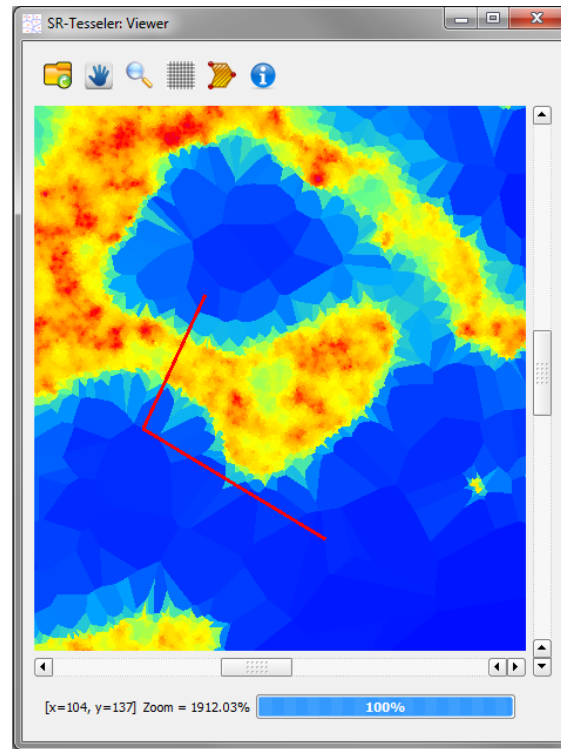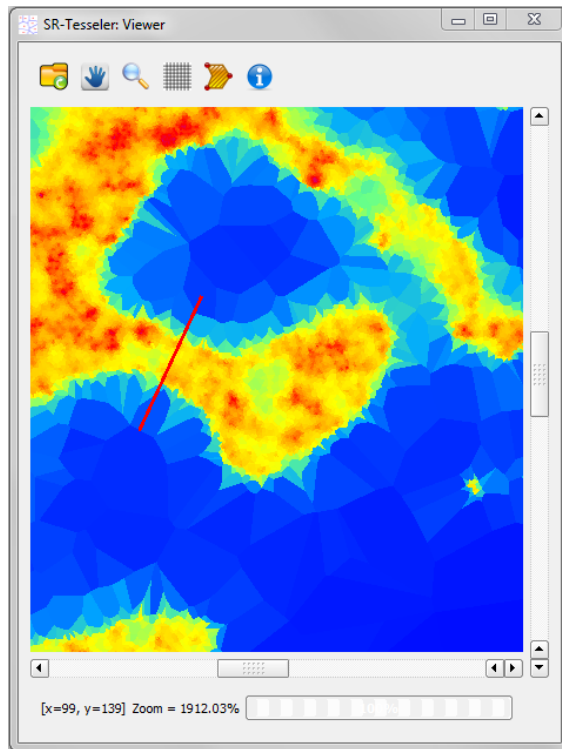- Select the hand icon to scroll the image

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
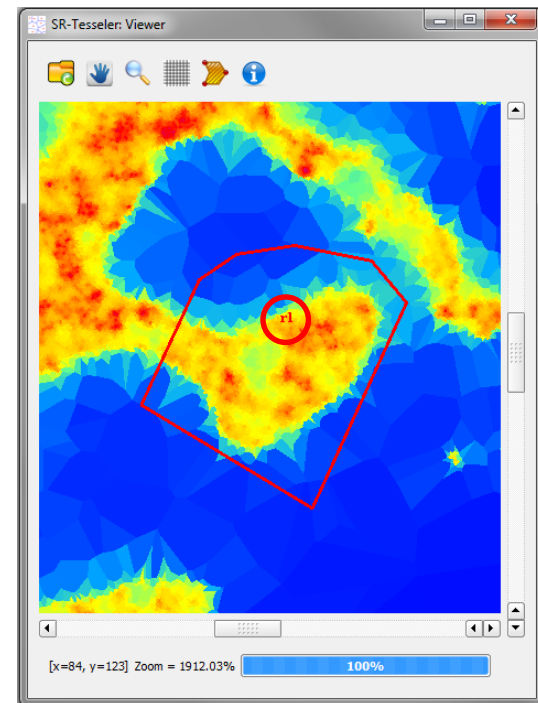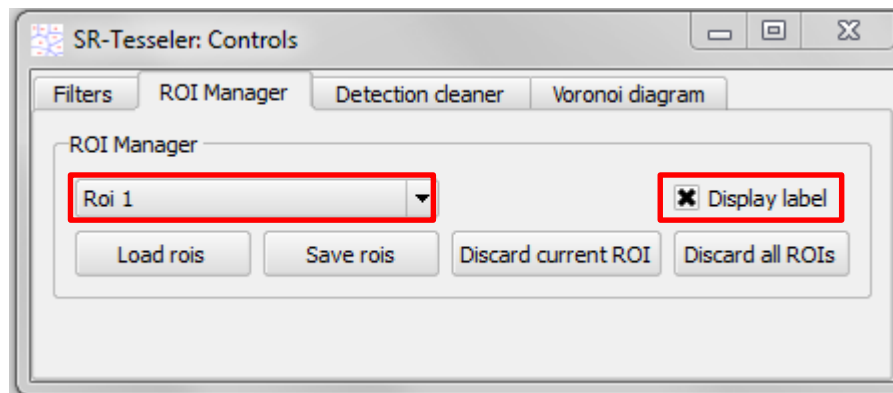- To add a ROI, click on the Polygon icon
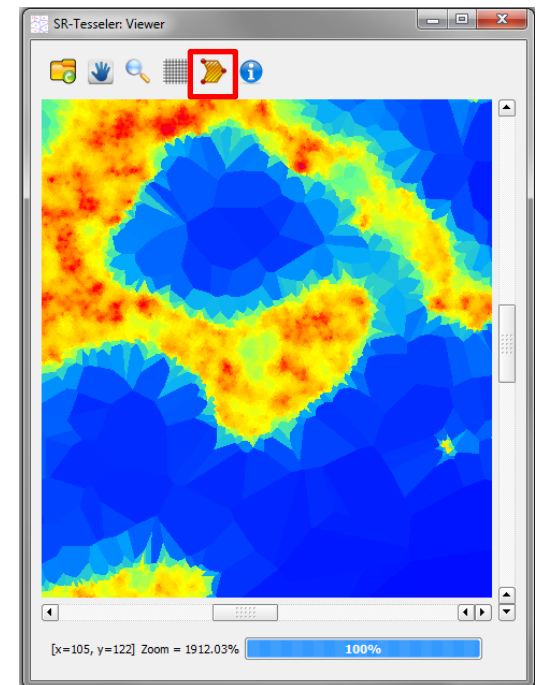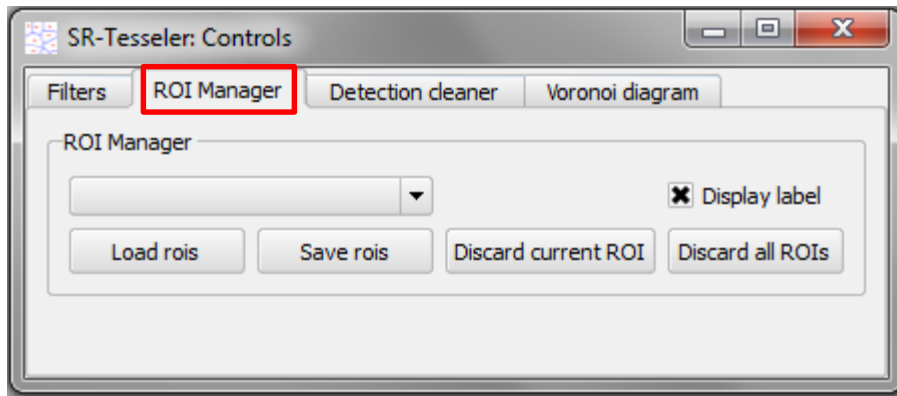
SR-Tesseler: ROI Manager (2/3)

- 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
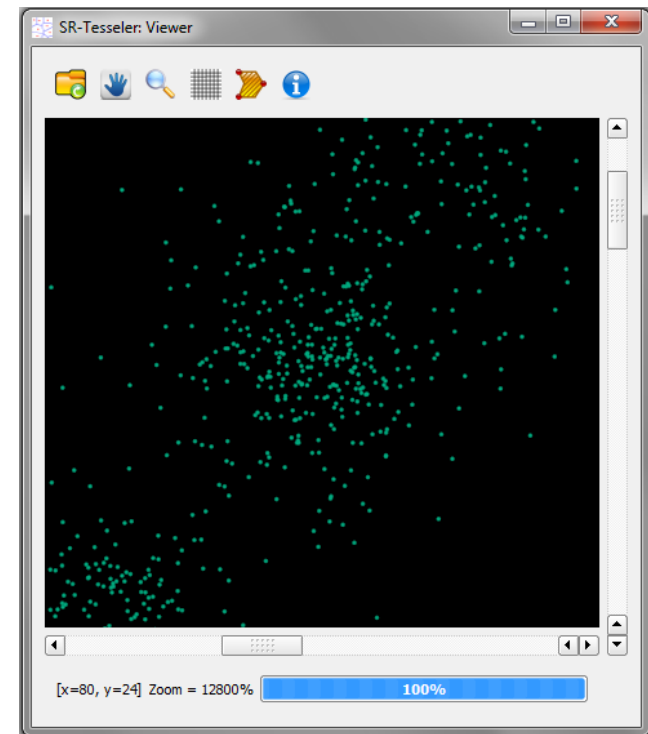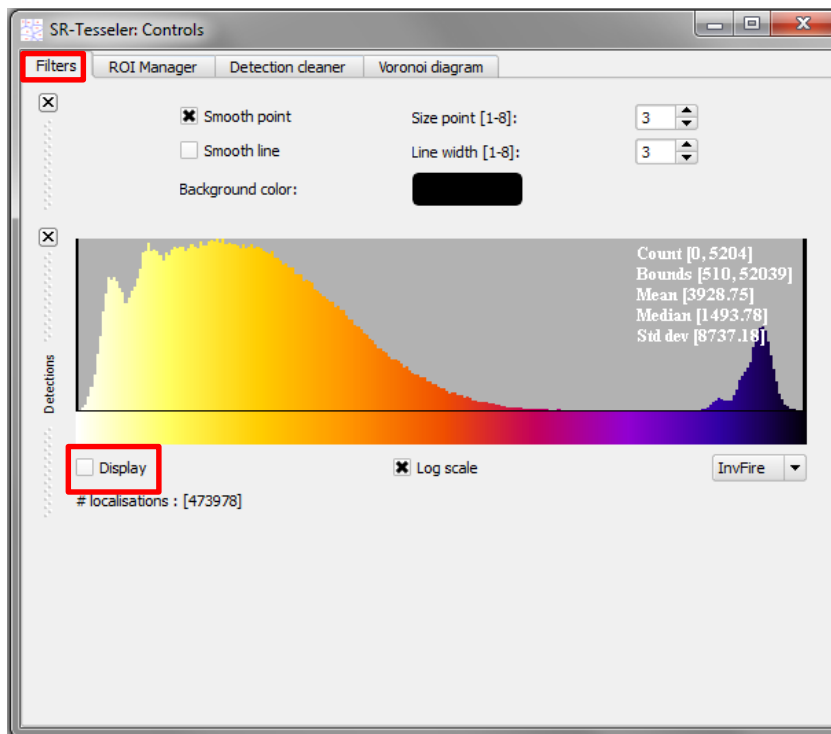
- Click the « Cleaning process » button
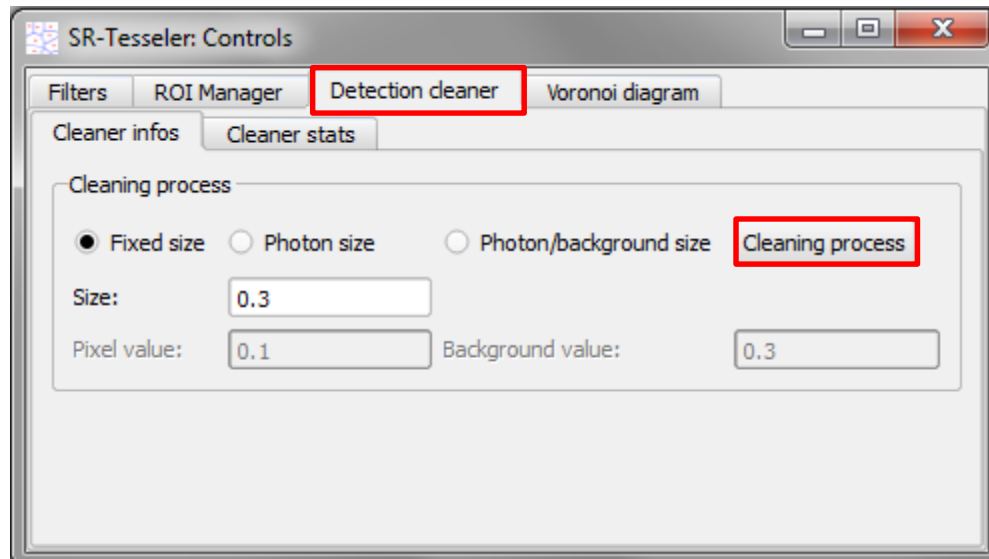
SR-Tesseler: blinking correction (2/4)

- The blinking-corrected detection dataset is created
- The two detection sets, initial and corrected, can be displayed by checking the Display checkboxes
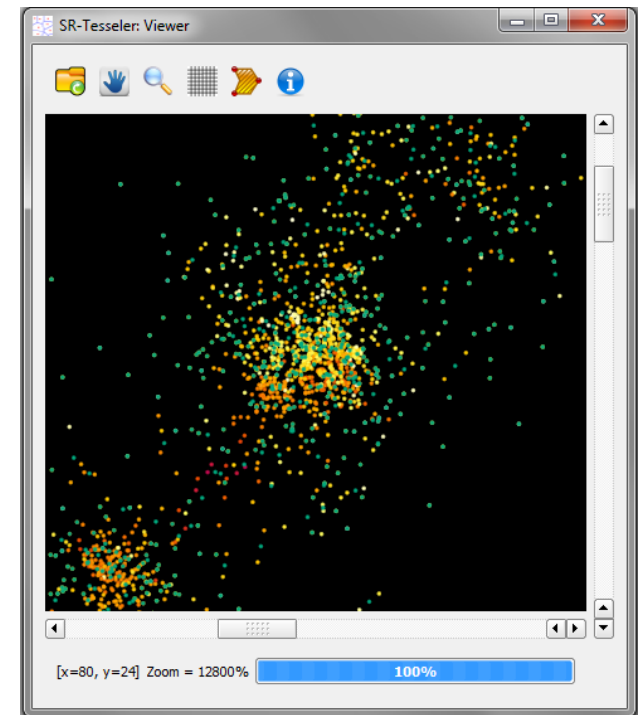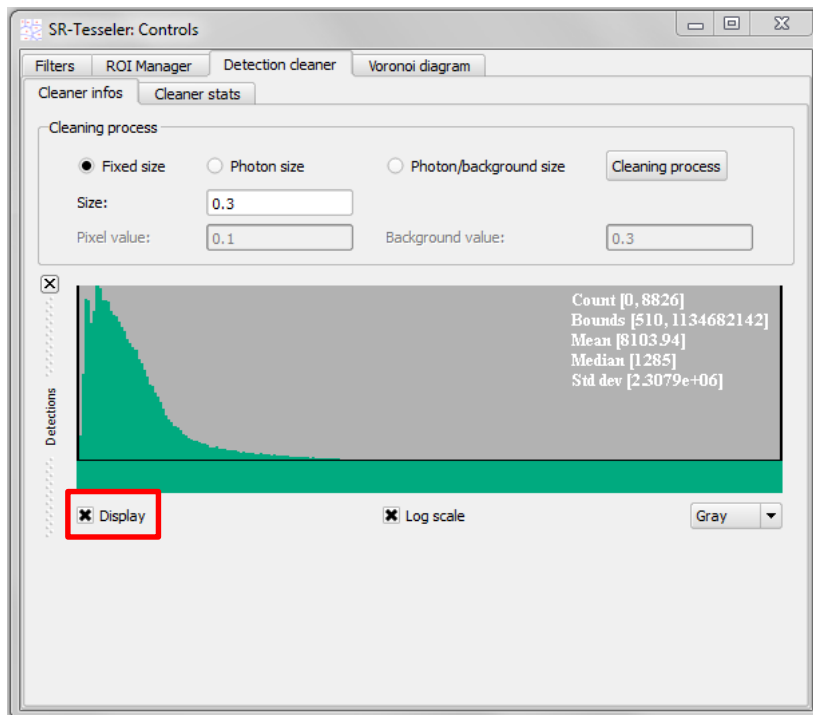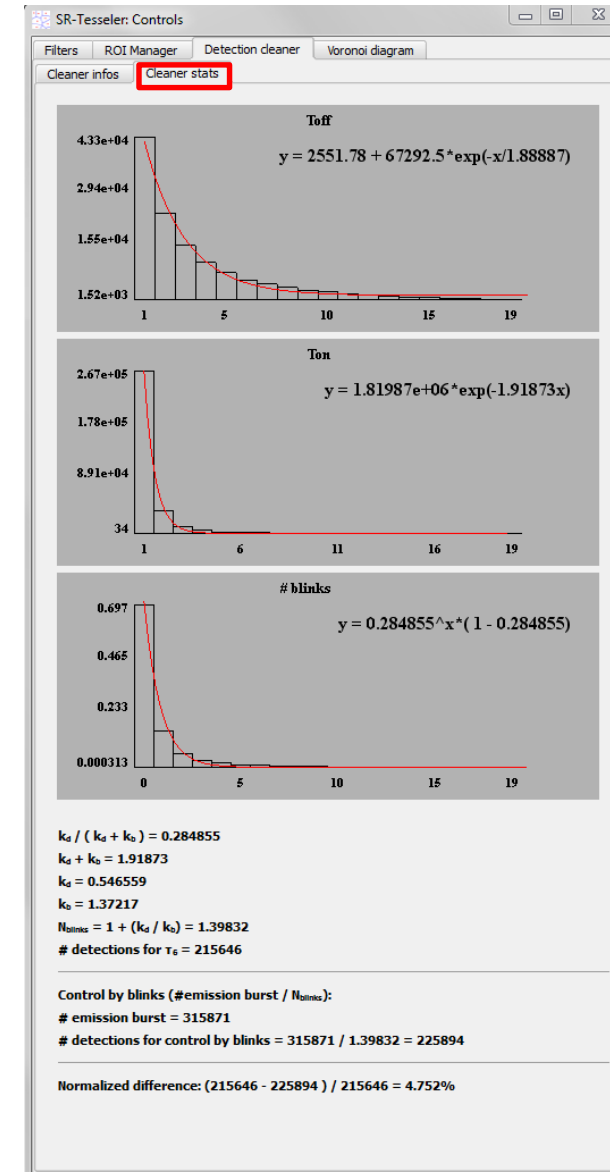
SR-Tesseler: blinking correction (3/4)

- 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*
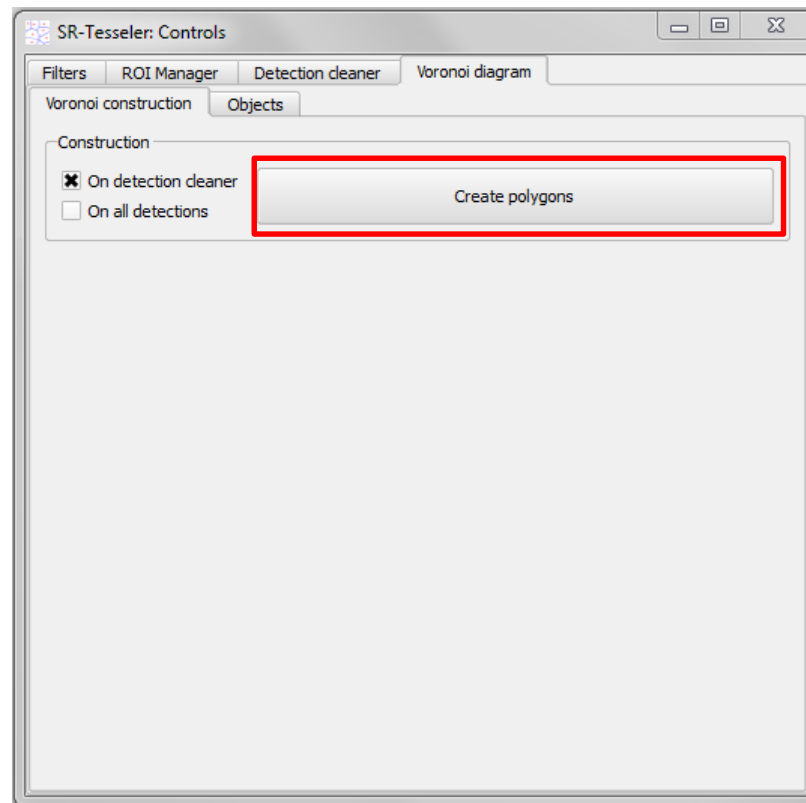
SR-Tesseler: Voronoï diagram (1/3)

- The Voronoï diagram can be computed either on the initial detection dataset, or on the blinking-corrected one (if exists)
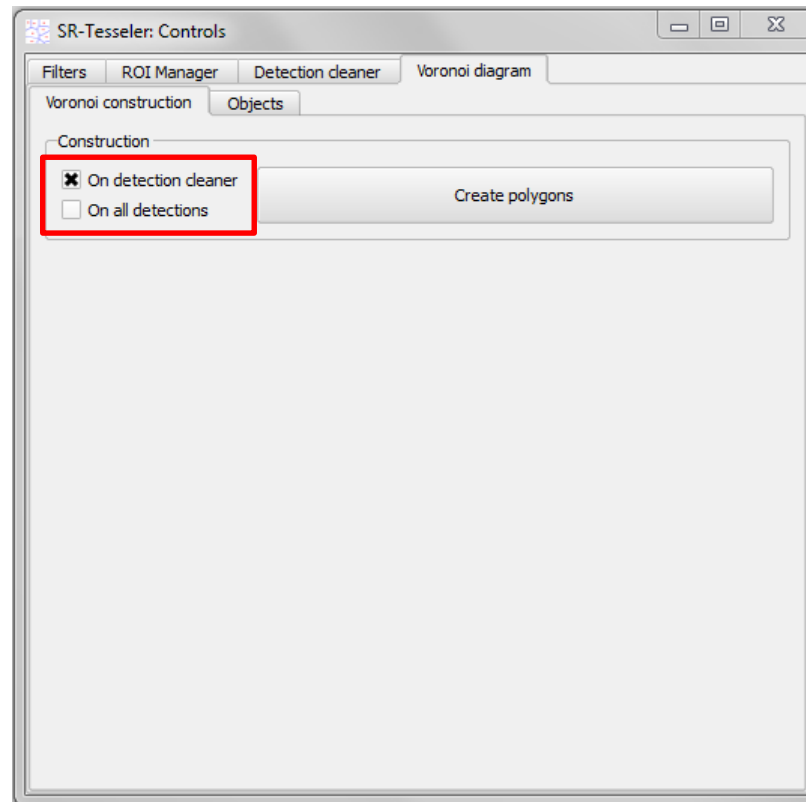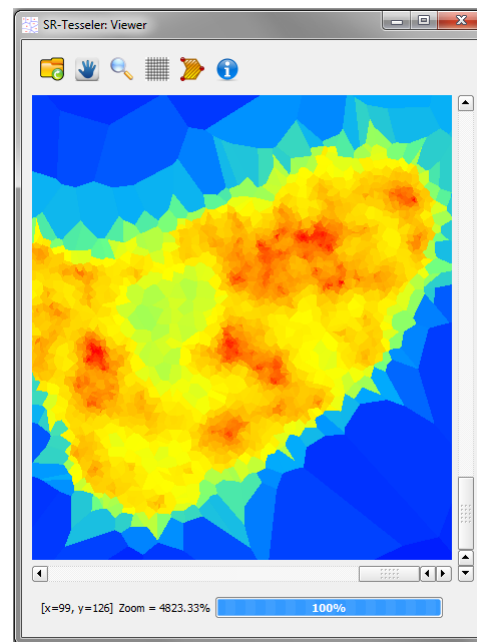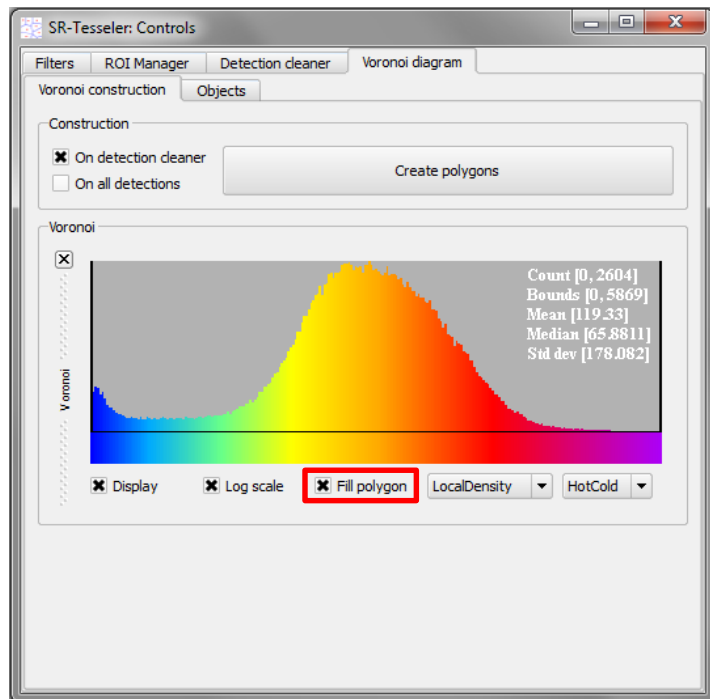
SR-Tesseler: Voronoï diagram (2/3)

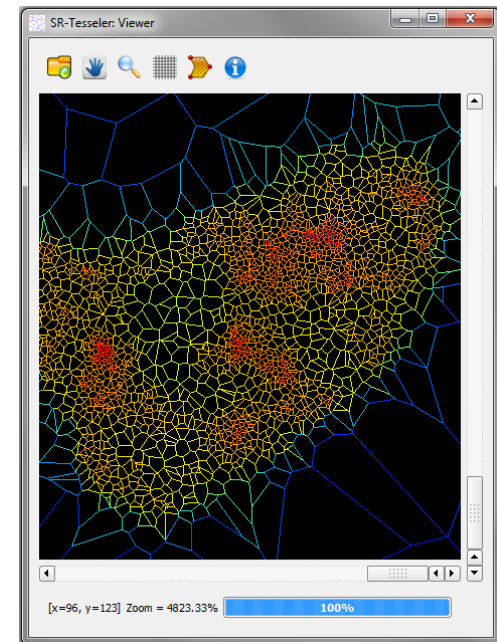- Click the « Create polygons » button from the « Voronoï diagram » tab to generate the tesselation

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
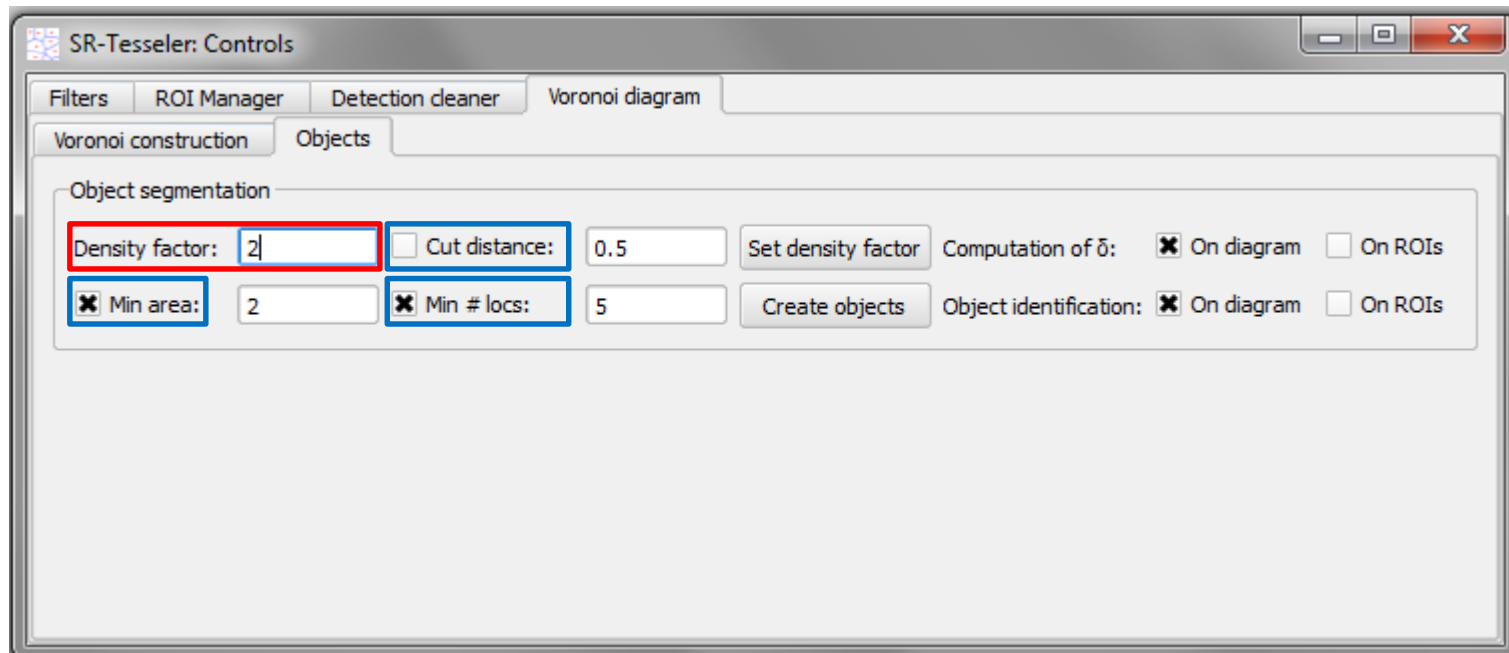
SR-Tesseler: Object creation (2/7)

- 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)
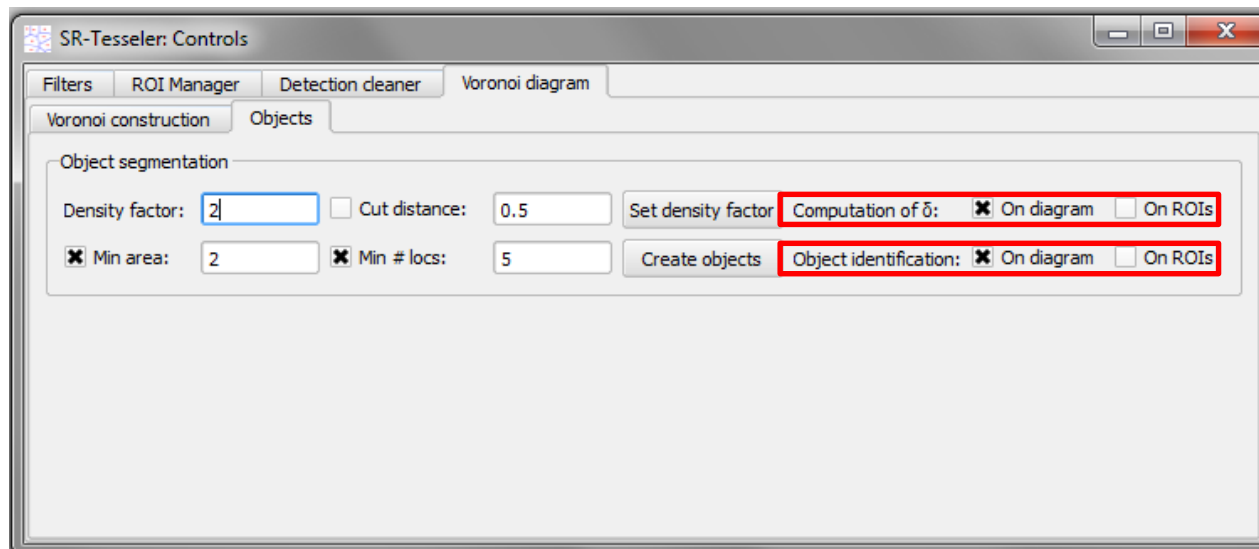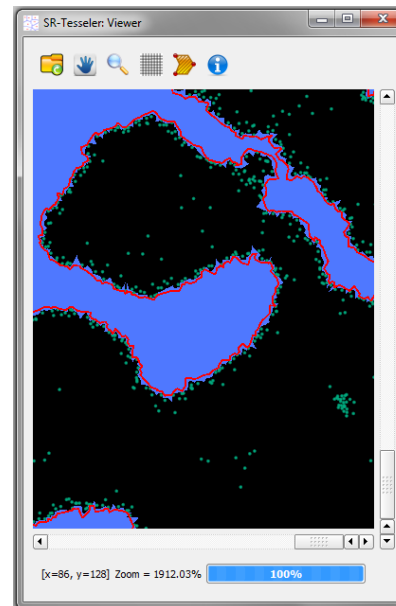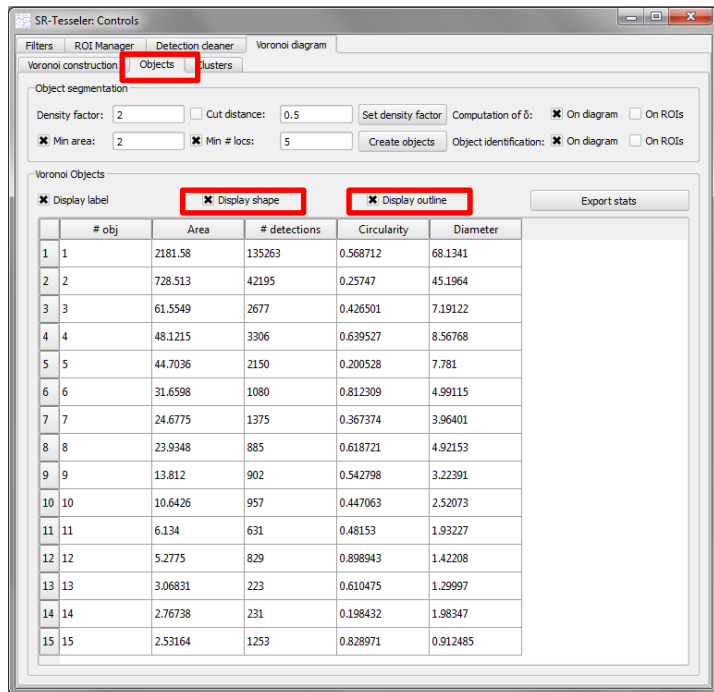
SR-Tesseler: Object creation (3/7)

- Click « Set density factor » then « Create objects » to compute the object
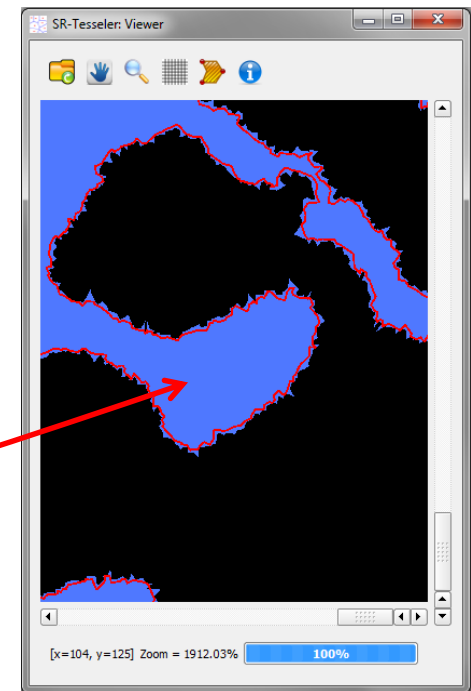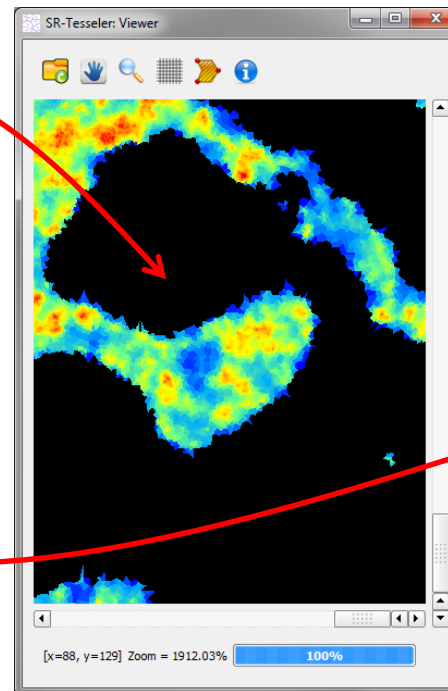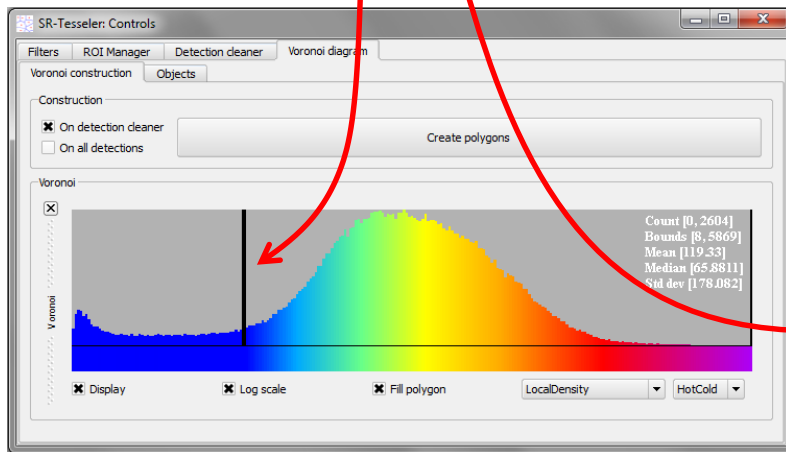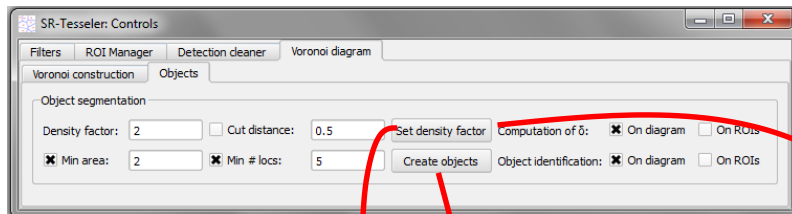
SR-Tesseler: Object creation (4/7)

- 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
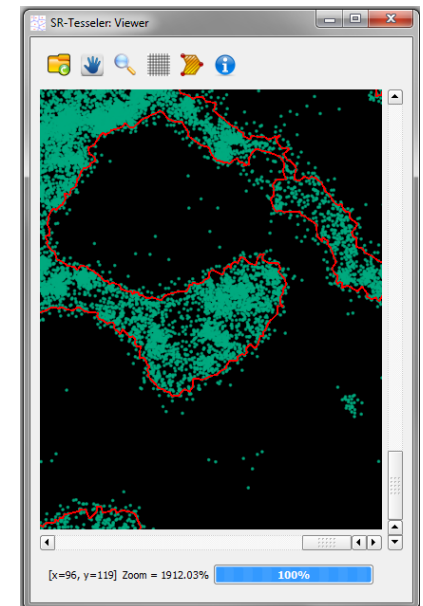
« Display outline » checked

SR-Tesseler: Object creation (5/7)

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

SR-Tesseler: Object creation (6/7)

- Example for object creation with ROIs
  - Create a ROI
  - Select « On ROIs » for the object creation
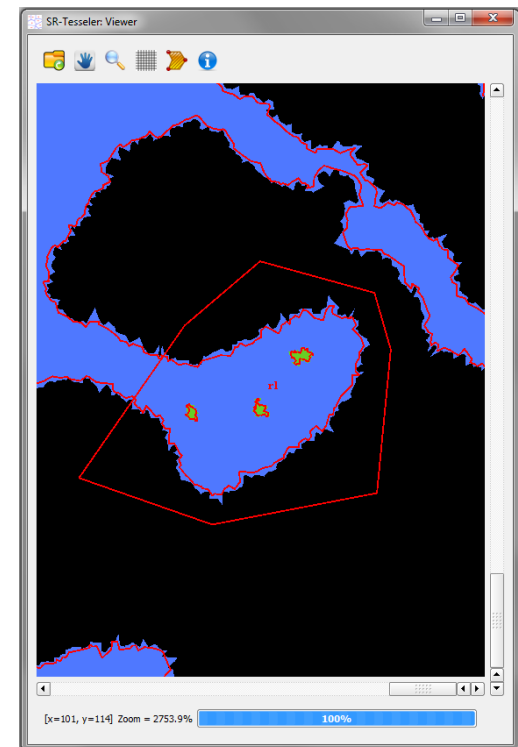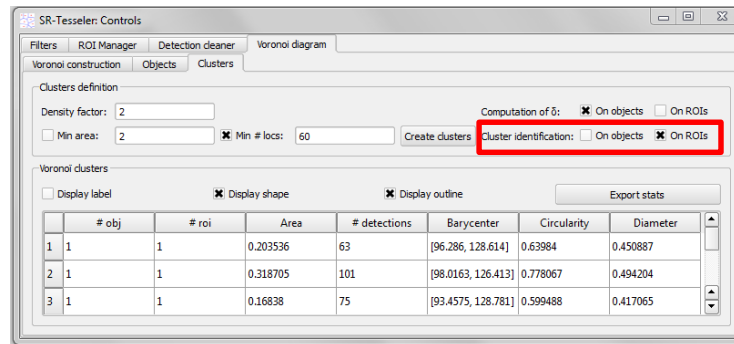 - Click « Set density » then « Create objects »
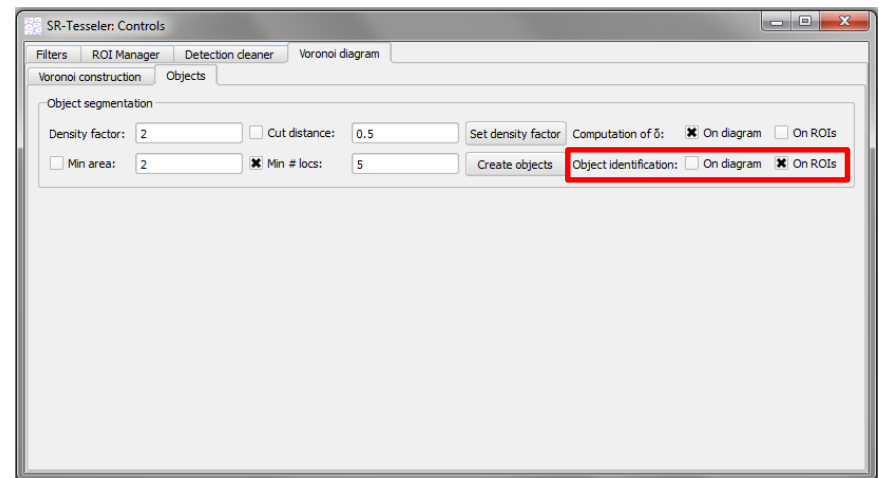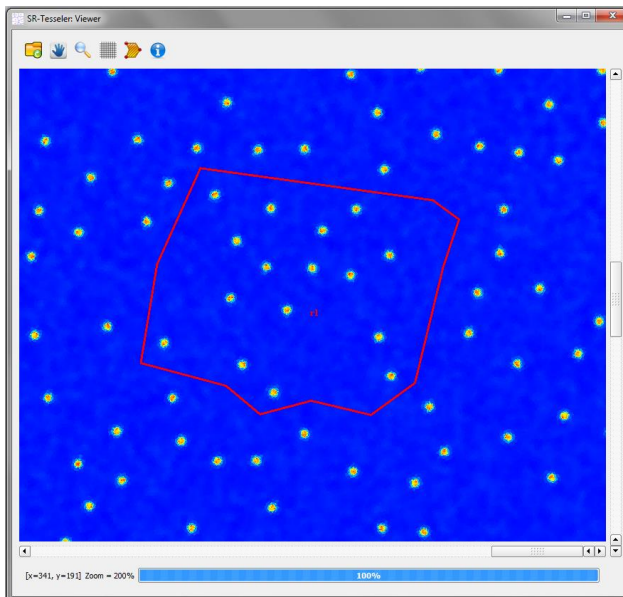
SR-Tesseler: Object creation (7/7)

- Example for object creation with ROIs
    - Create a ROI
    - Select « On ROIs » for the object creation
    - Click « Set density » then « Create objects »
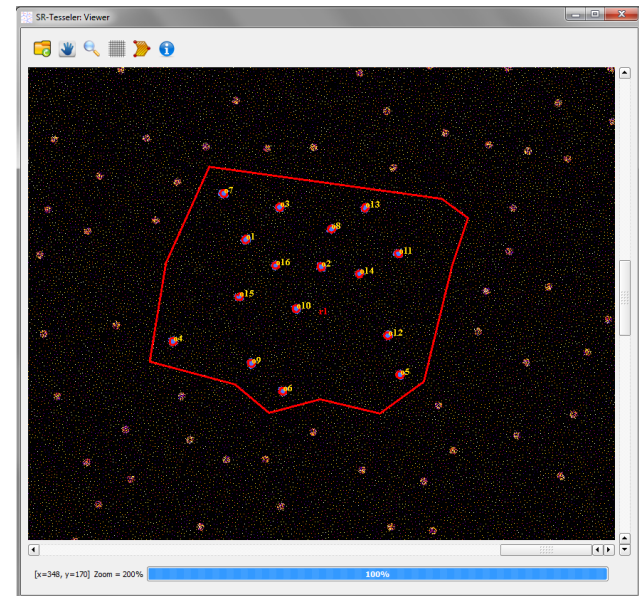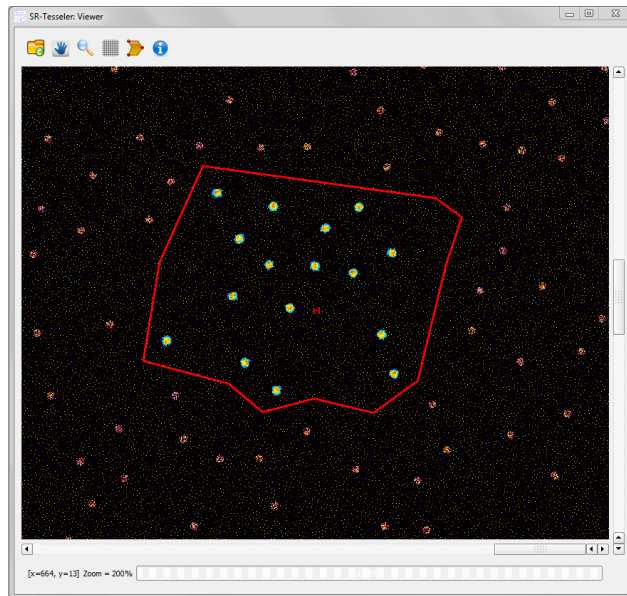
SR-Tesseler: Cluster creation (1/6)

- 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 pixel^2) and number of detections per cluster
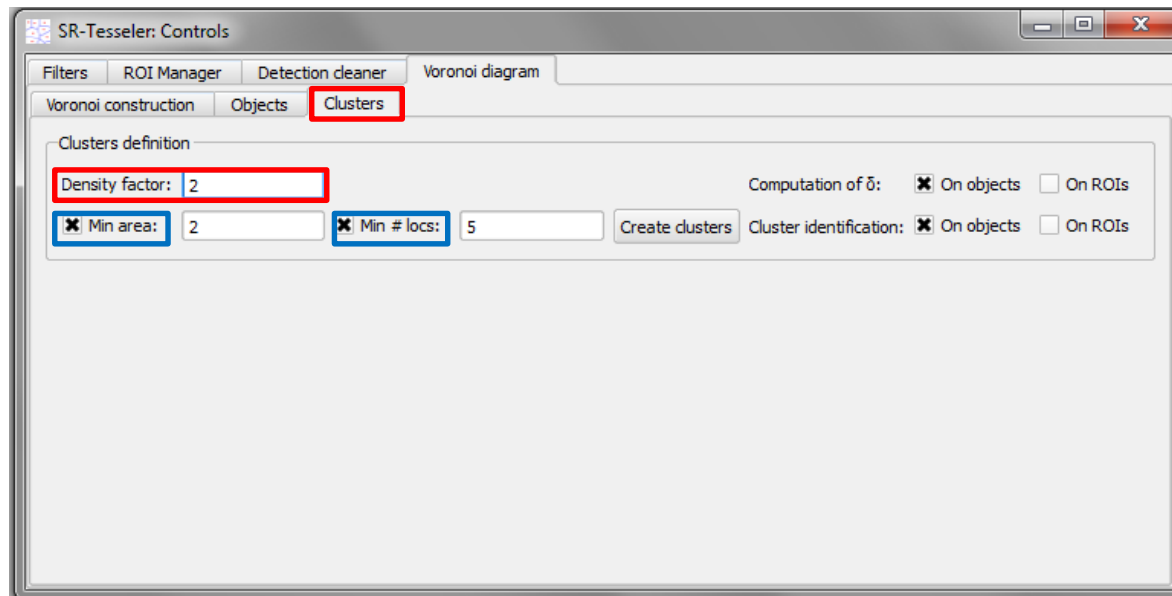
SR-Tesseler: Cluster creation (2/6)

- δ can be computed for each object or globally on all the objets inside the ROIs

- Clusters can be created for each object or only on the objects being inside the ROIs (example in slide 6/6)
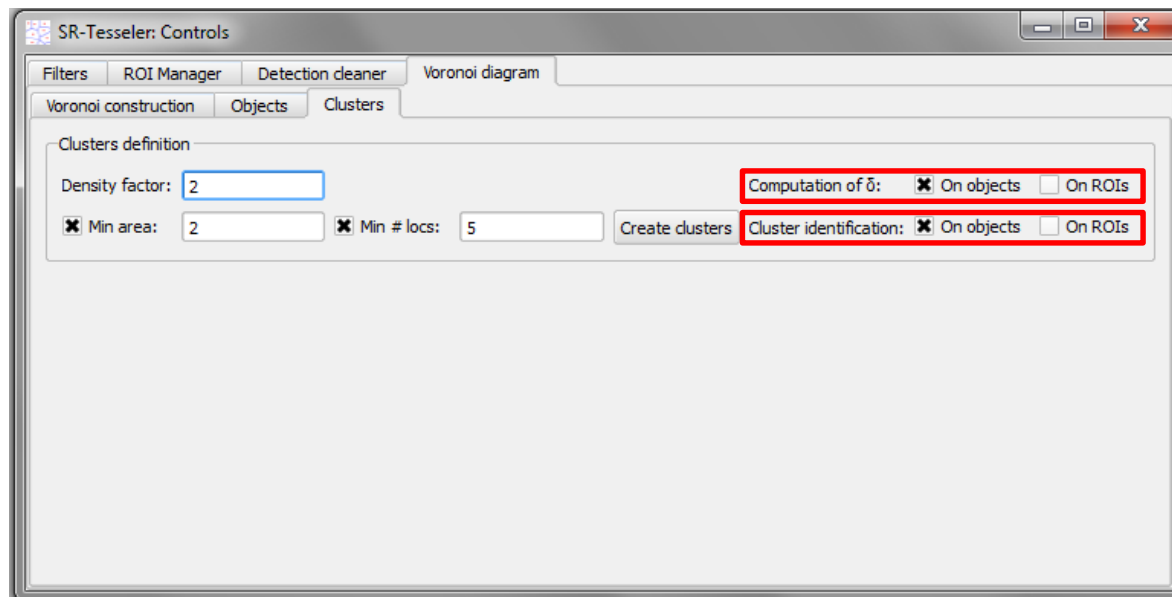
SR-Tesseler: Cluster creation (3/6)

- Click on « Create clusters » to compute clusters
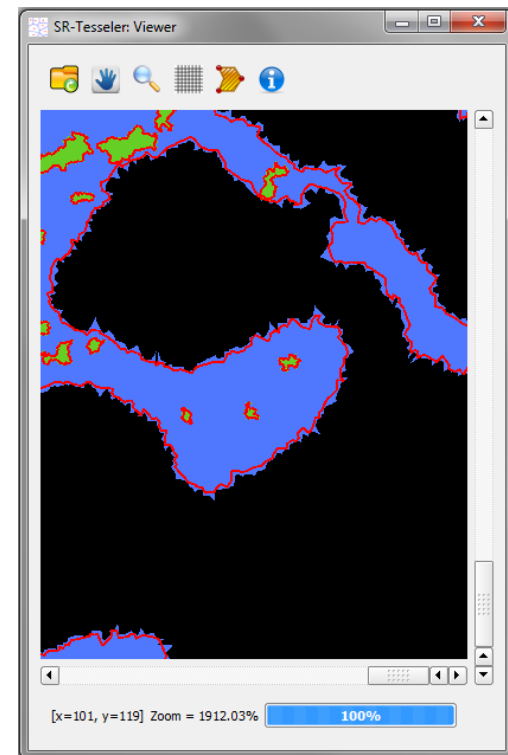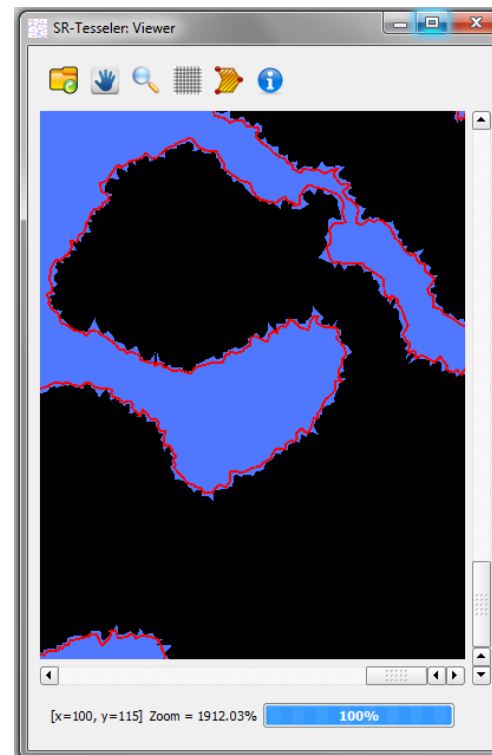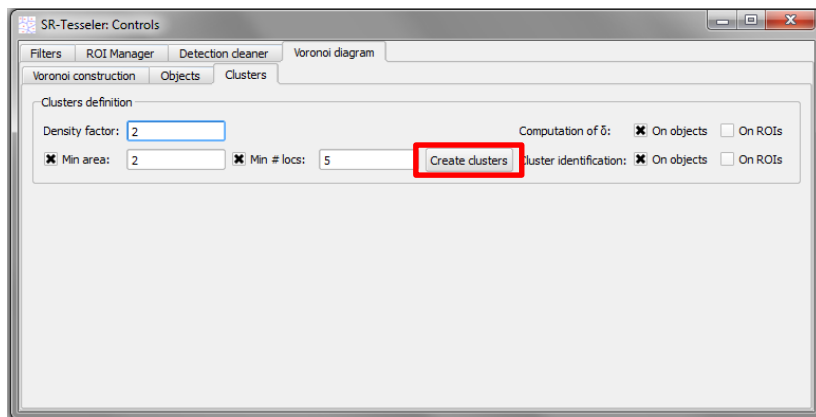
SR-Tesseler: Cluster creation (4/6)

- 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
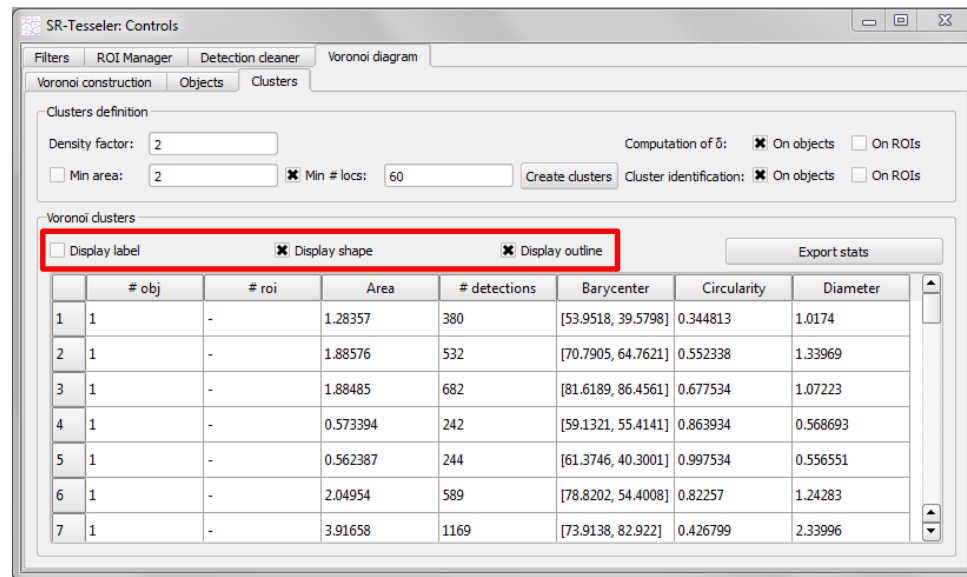
SR-Tesseler: Cluster creation (5/6)

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

SR-Tesseler: Cluster creation (6/6)

- Example of clusters creation with "On ROIs" selected
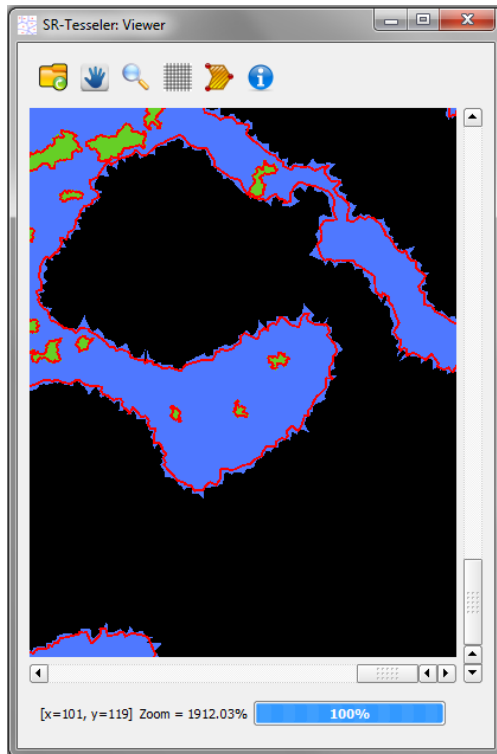
Data format (1/3)

- In the data directory, one file describing the localizations is needed.

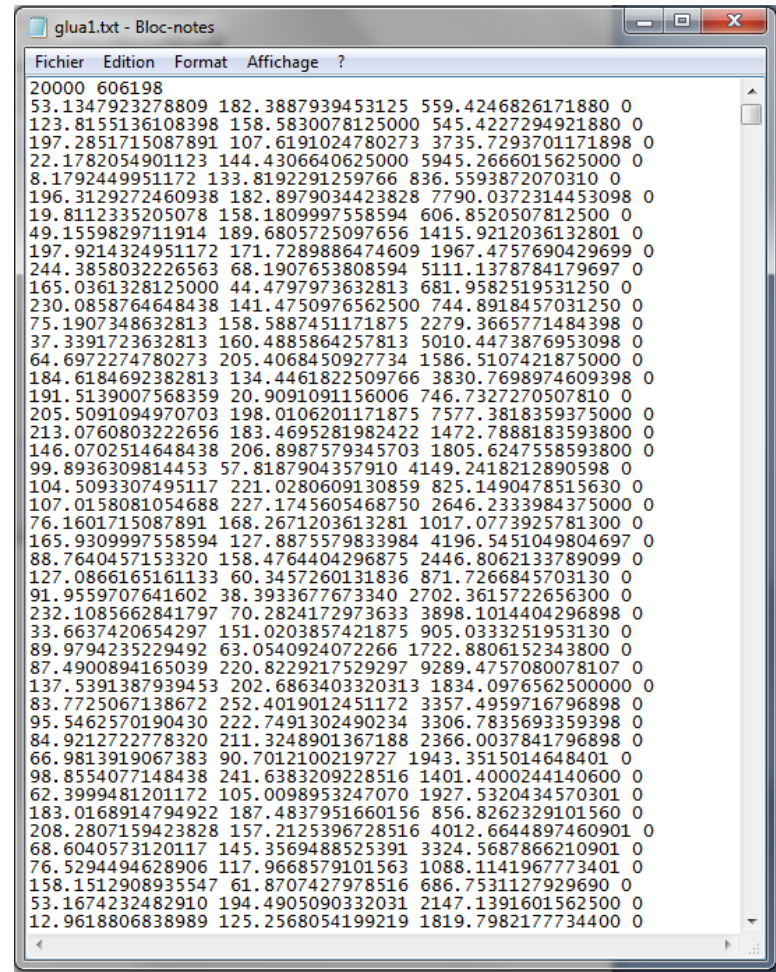- Two formats are possible
  - An ASCII txt file
  - A CSV file
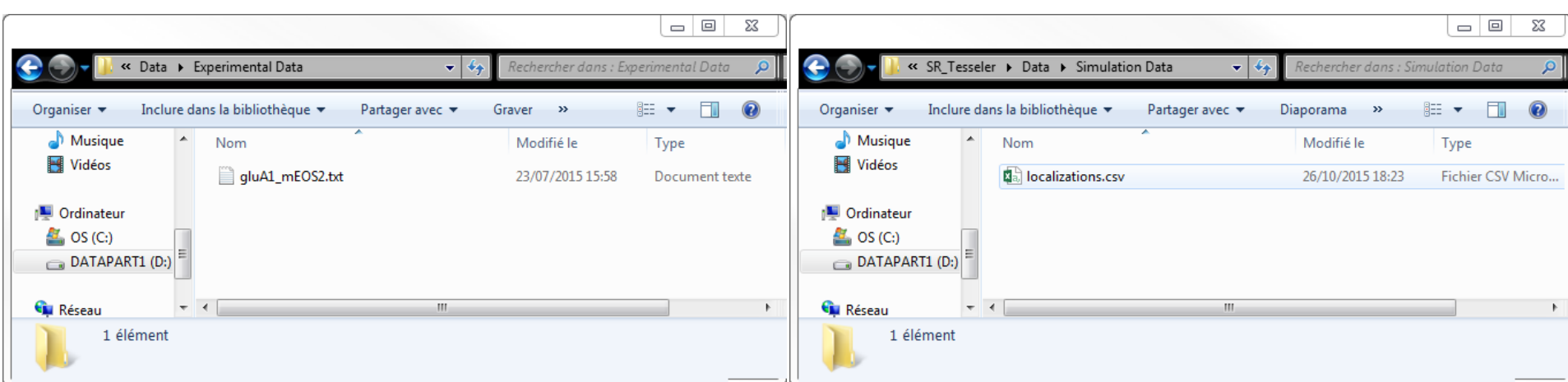
Data format (2/3)

- Detection ASCII file format

Header: [Nb planes, Nb detections]

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

Data format (3/3)

- ## CSV file format

Header line
sigma is optional

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

Known limitations (1/2)

- For the 32 bits windows environment
  - Internal memory used by SR-Tesseler is limited
  - Maximum number of localizations for the Voronoï diagram creation is ≈ 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.