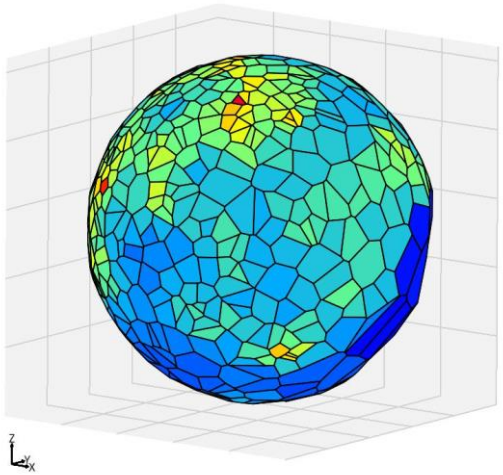
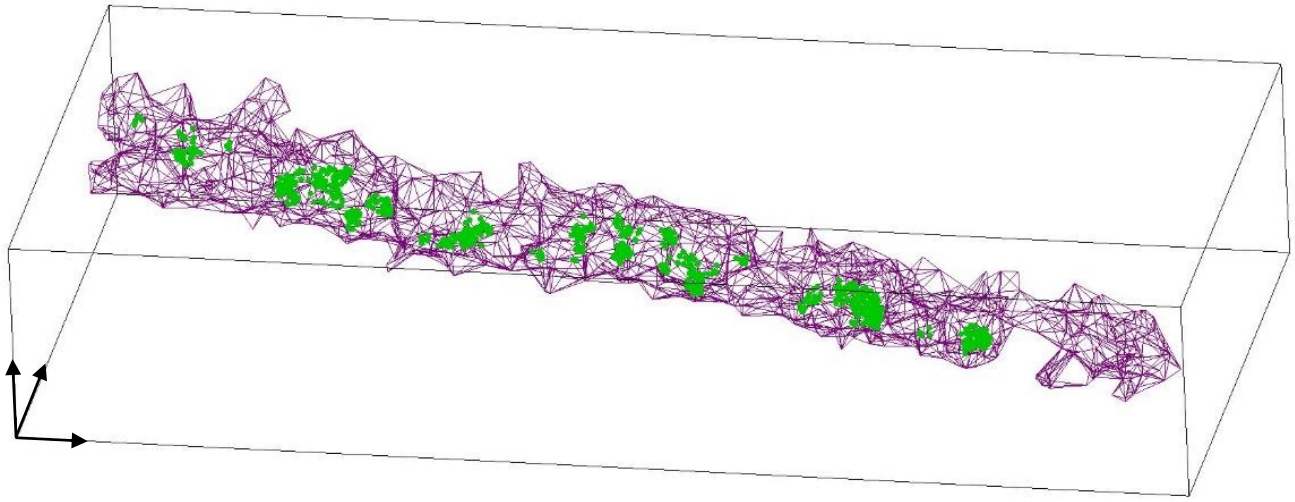
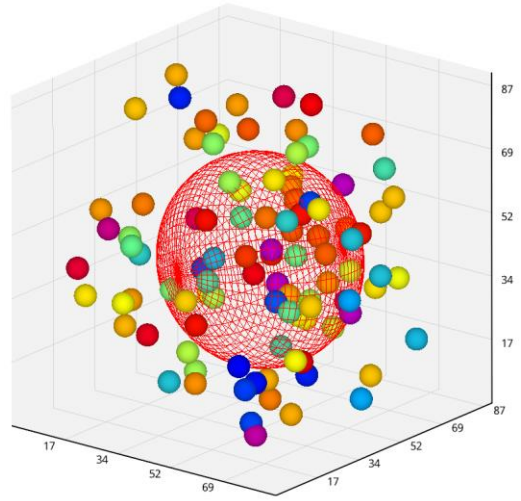
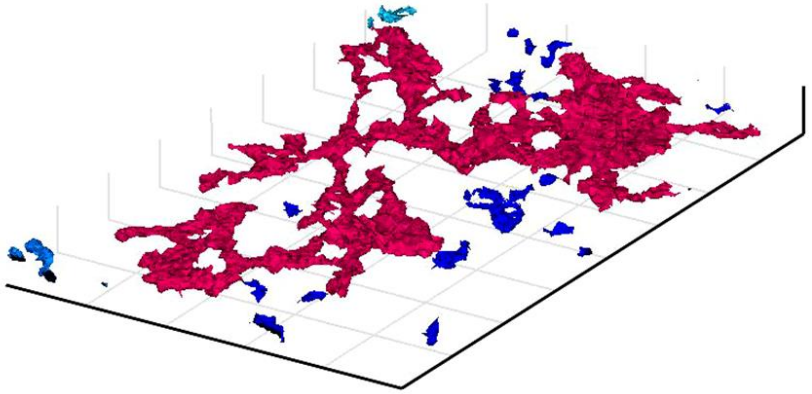
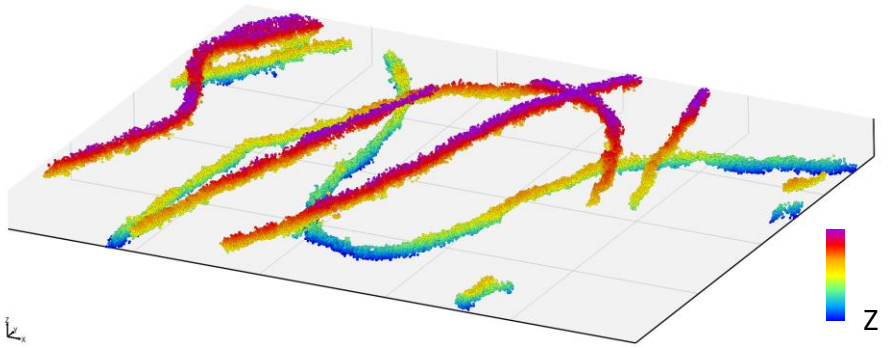








PoCA manual



Florian Levet

Quantitative Imaging of the Cell – Sibarita's team

Interdisciplinary Institute for Neuroscience

CNRS UMR 5297, University of Bordeaux

https://github.com/flevet/PoCA
https://github.com/flevet/PoCA/releases
https://github.com/flevet/PoCA/issues

flevet @f_levet

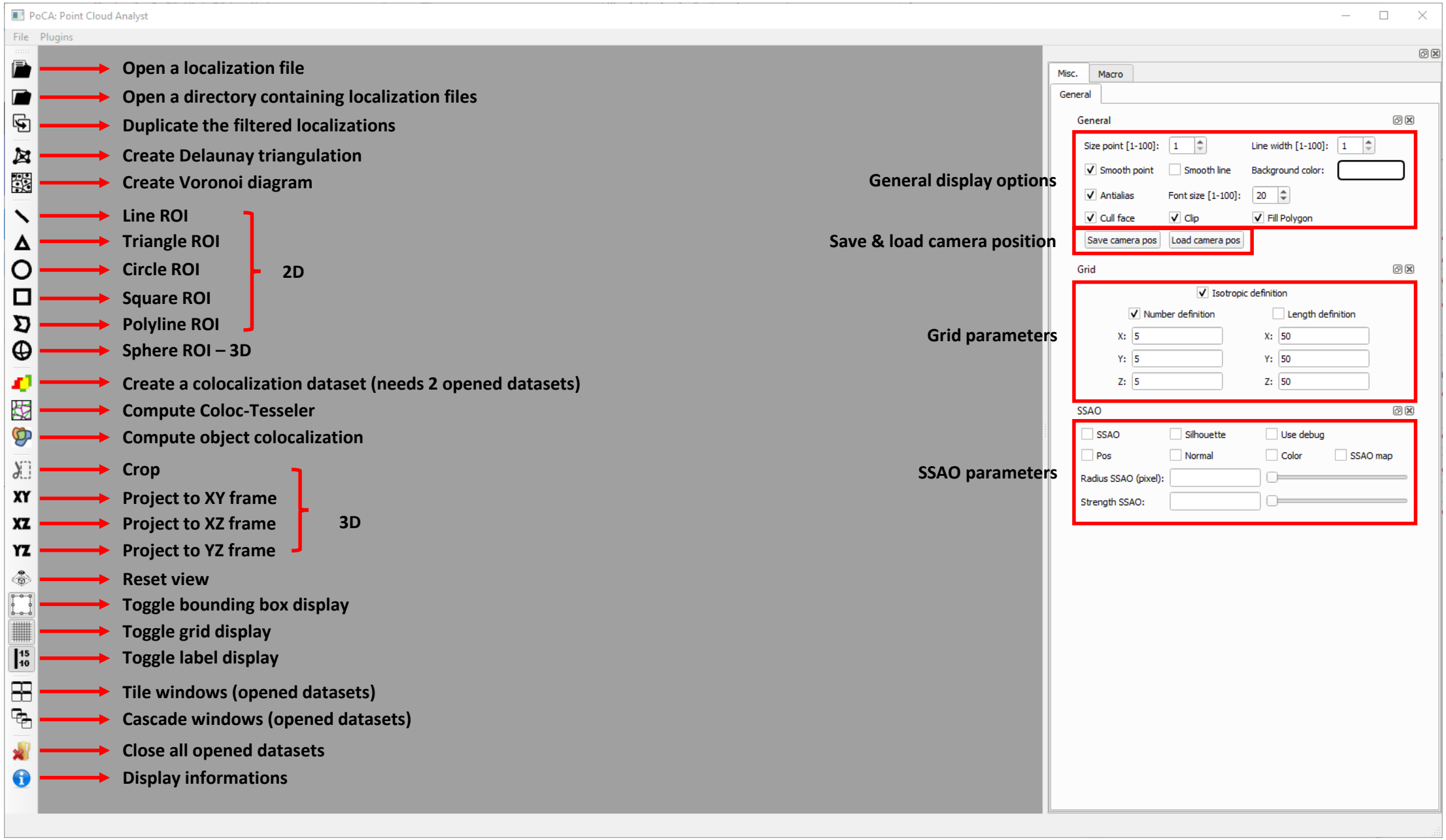
Requirements when using the Windows binaries

- If upon executing poca.exe Windows asks for dlls such as "VCRUNTIME140_1.dll", you may need to install the "microsoft visual c++ 2019 redistributable package (x64)": https://docs.microsoft.com/en-GB/cpp/windows/latest-supported-vc-redist?view=msvc-160

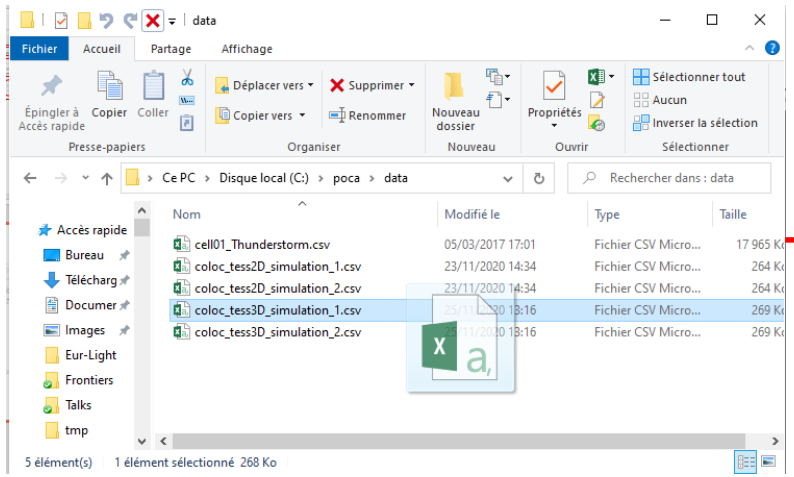
- For having access to the Voronoi 3D construction and the 3D visualization, you will need a NVidia card with the latest drivers installed as well as CUDA (10.2 for instance, may work with newest versions): https://developer.nvidia.com/cuda-10.2-download-archive

- For communication with Python, you will need to have installed Python 3.7.x (tested with Python 3.7.4)

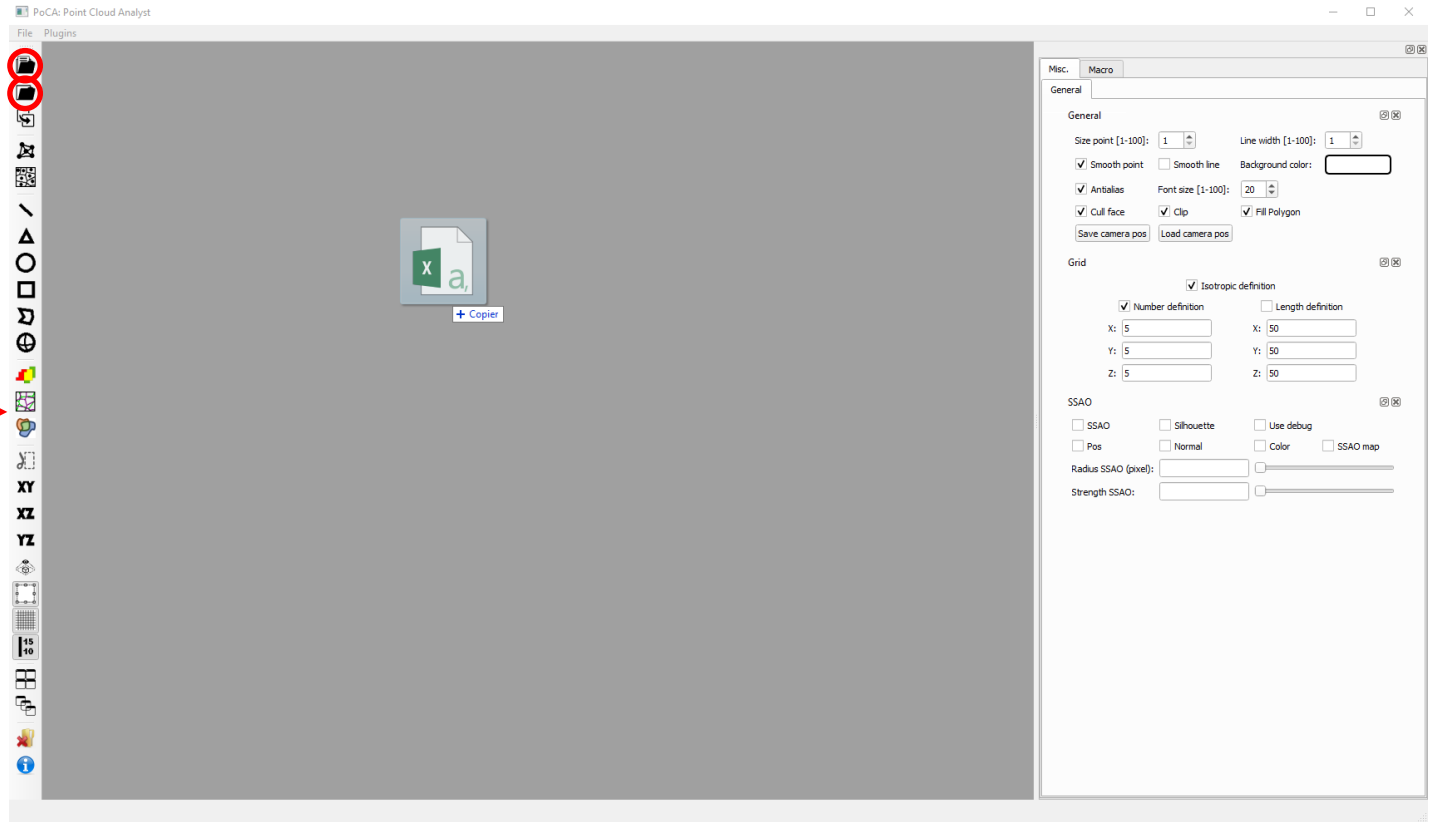
PoCA windows



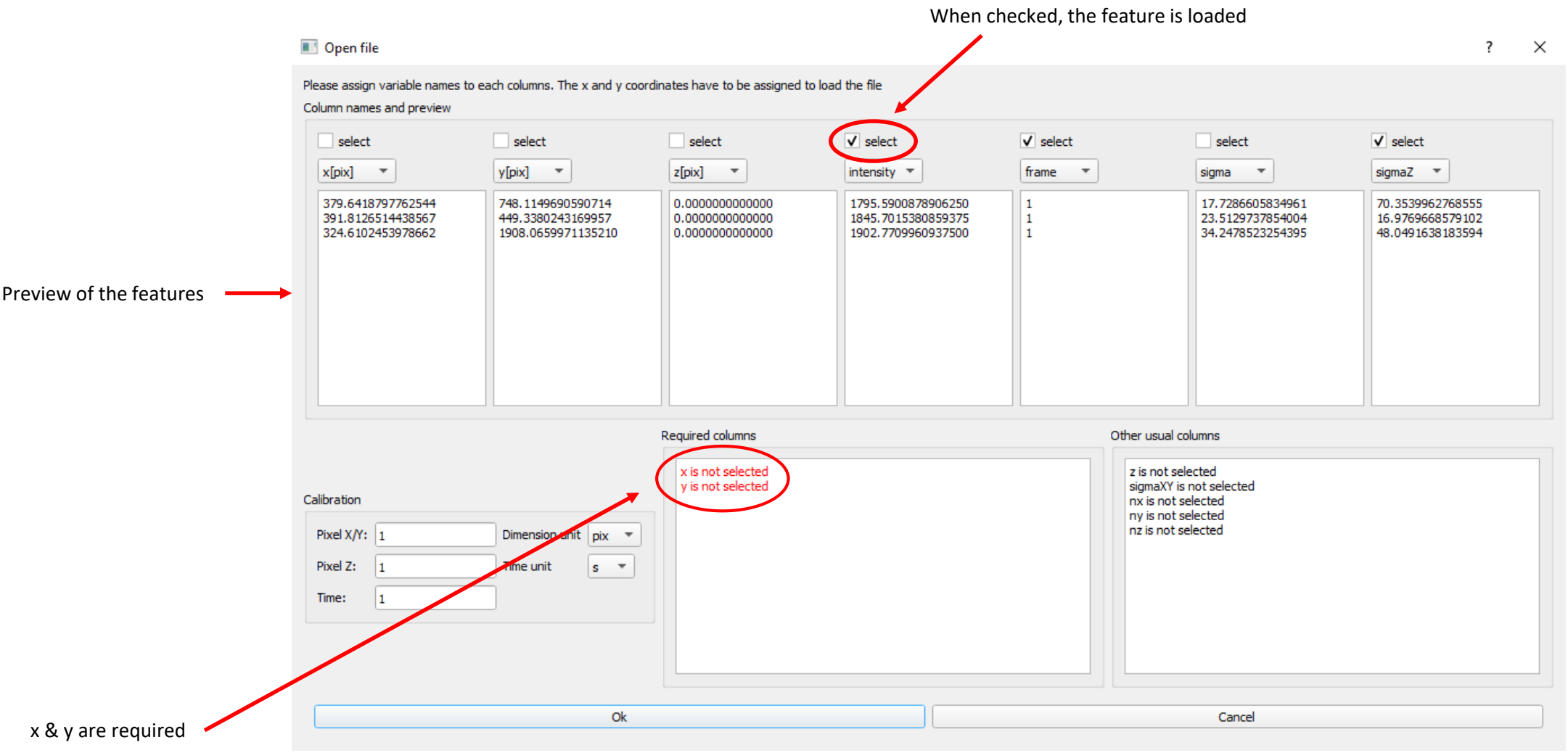
Open data: use file icon (choose the file), folder icon (open all locs files in the folder) or drag/drop csv file



Or

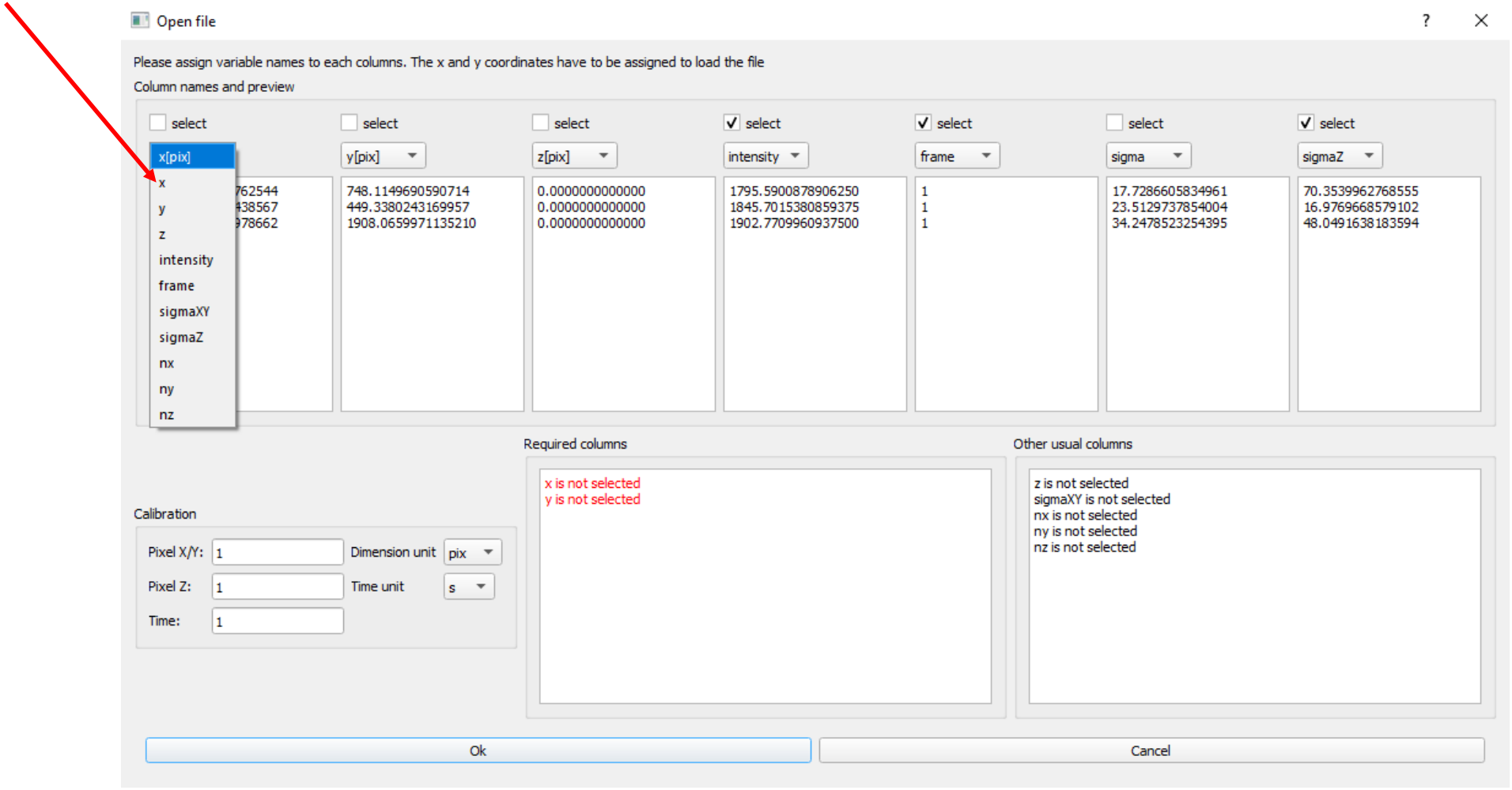


Open data: it will open a new windows where you will be able to choose the features to load



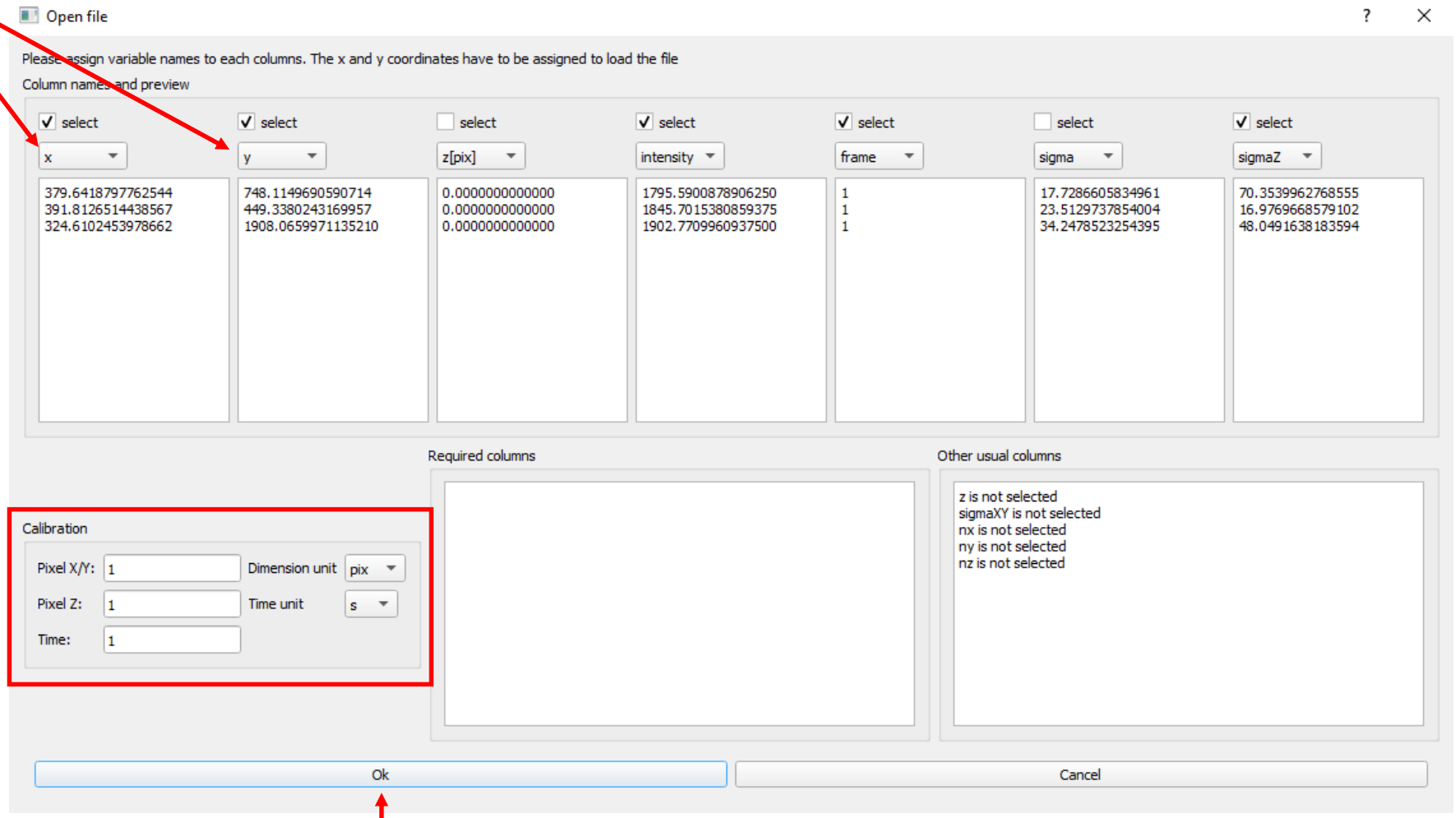
Open data: it will open a new windows where you will be able to choose the features to load

Click on the list to select x, and do the same for y



Open data: it will open a new windows where you will be able to choose the features to load

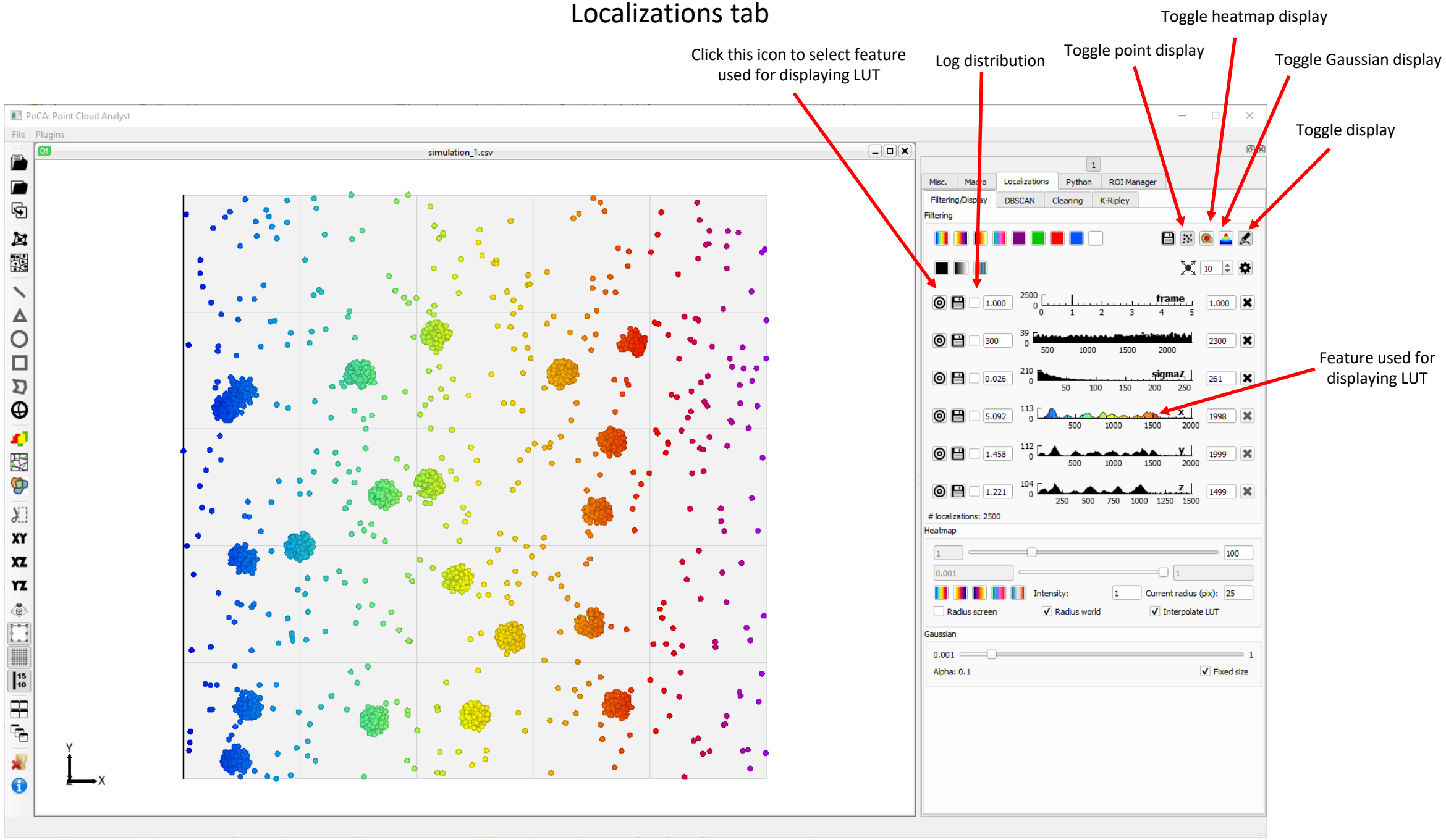
x & y are selected



You can calibrate the data

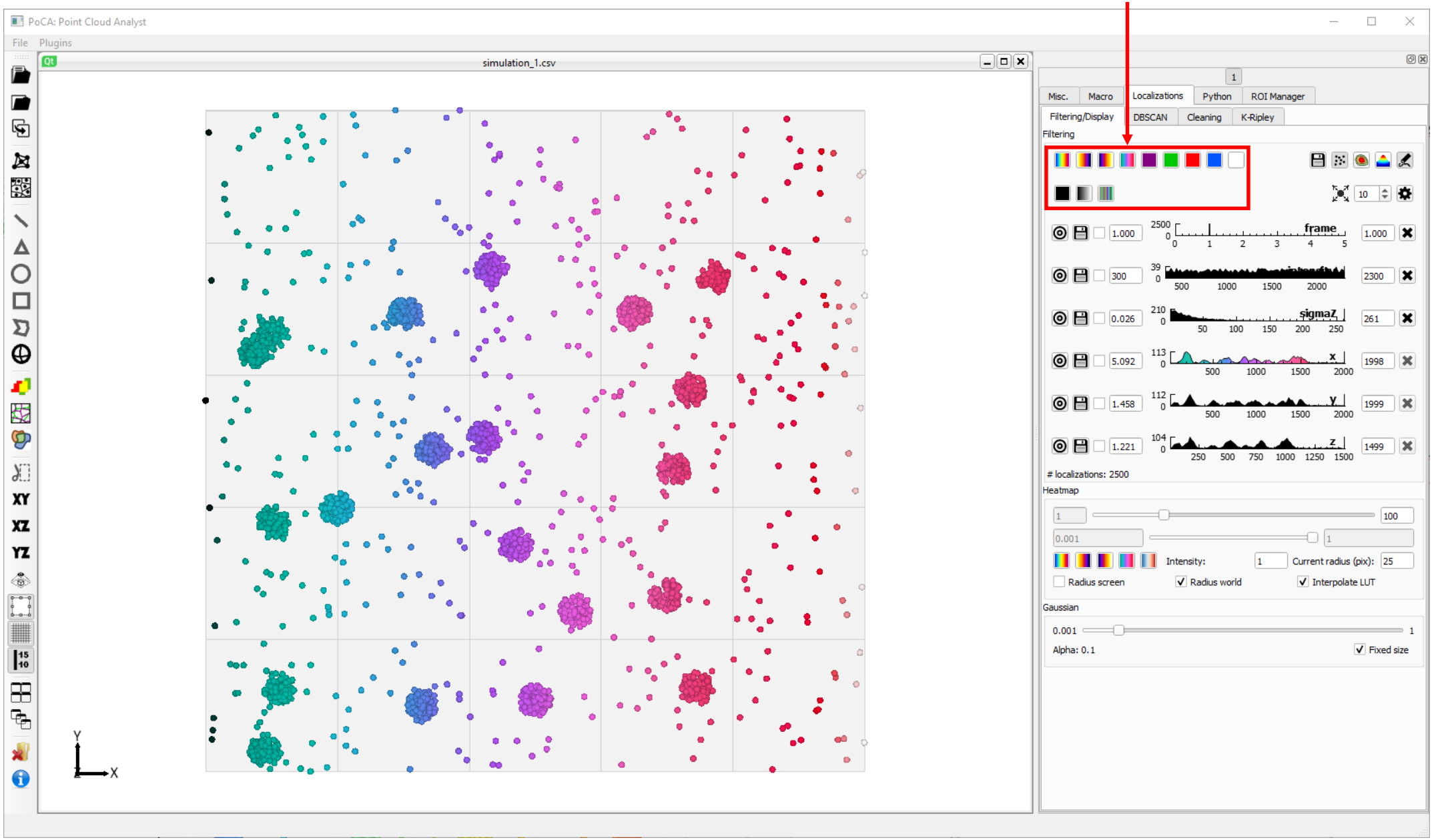
Click Ok to load the data with the selected features

Localizations tab

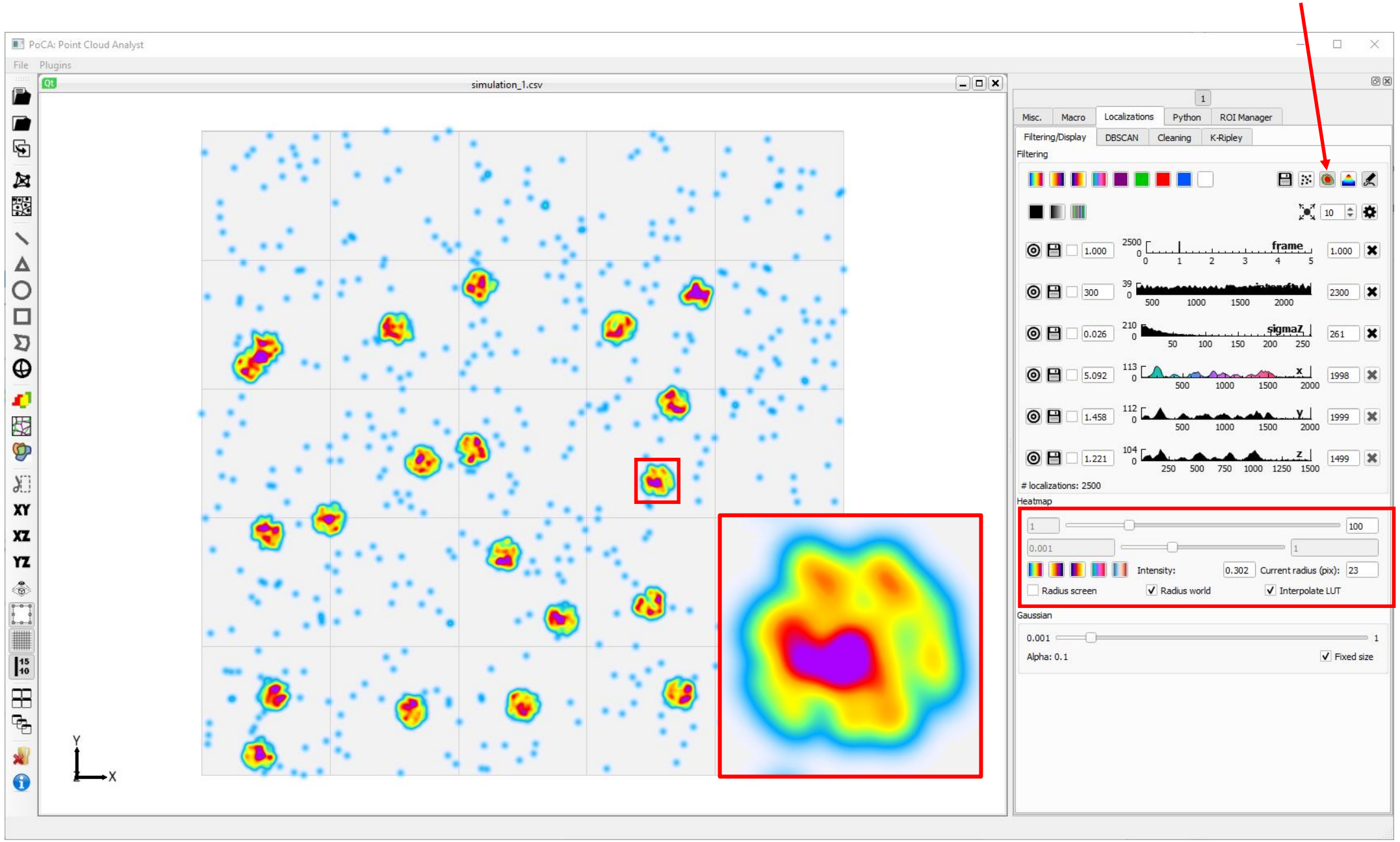


Selection of the intensity feature as LUT displaying with log distribution

Change easily which LUT is used by clicking on the icon

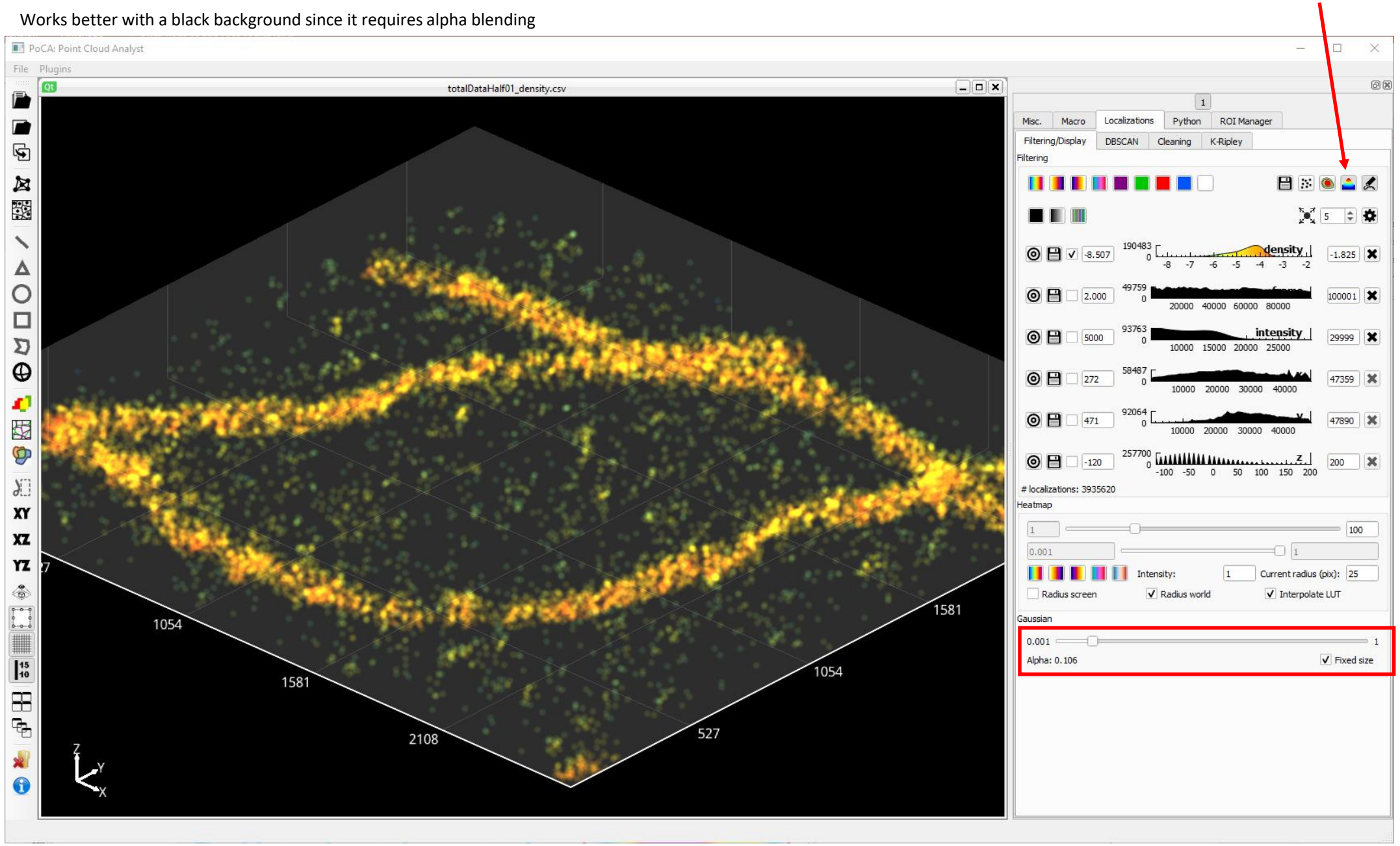


Heatmap rendering



Gaussian rendering

Works better with a black background since it requires alpha blending

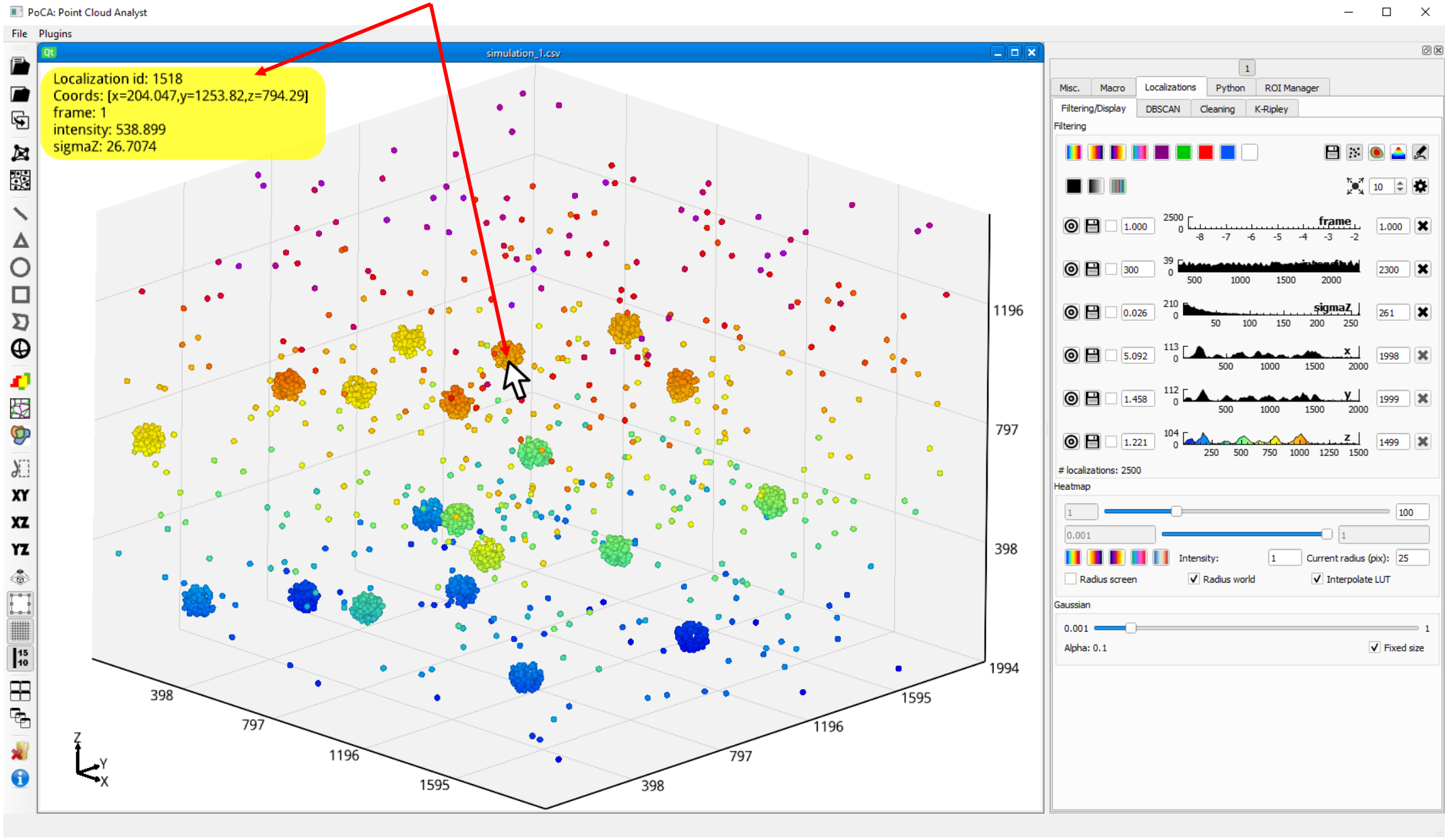


parameter

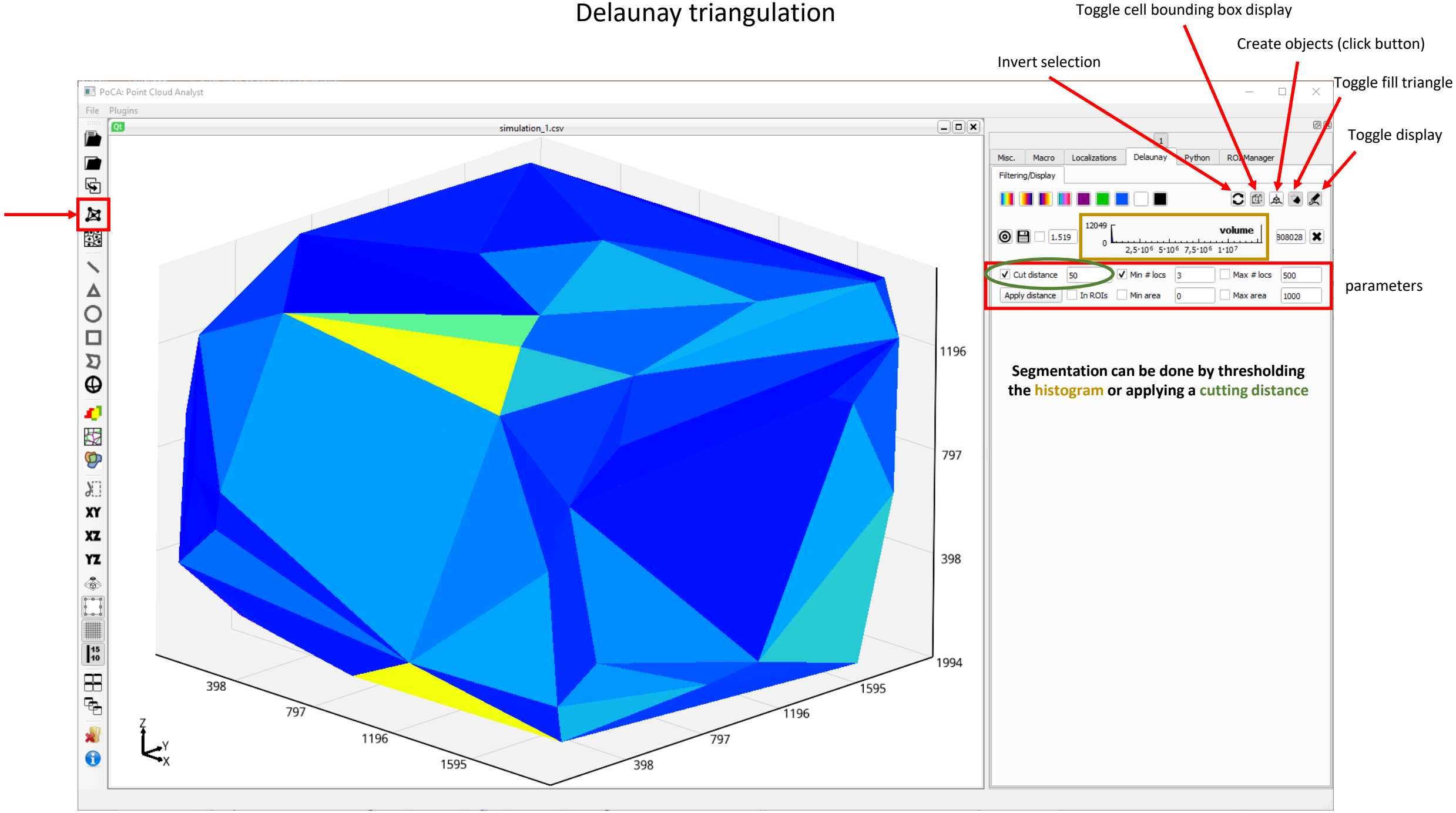
Picking

3D view
left click for rotation
Shift + left click for zoom
Middle click for translation
Wheel for zoom

Information on the localization under the mouse icon are displayed

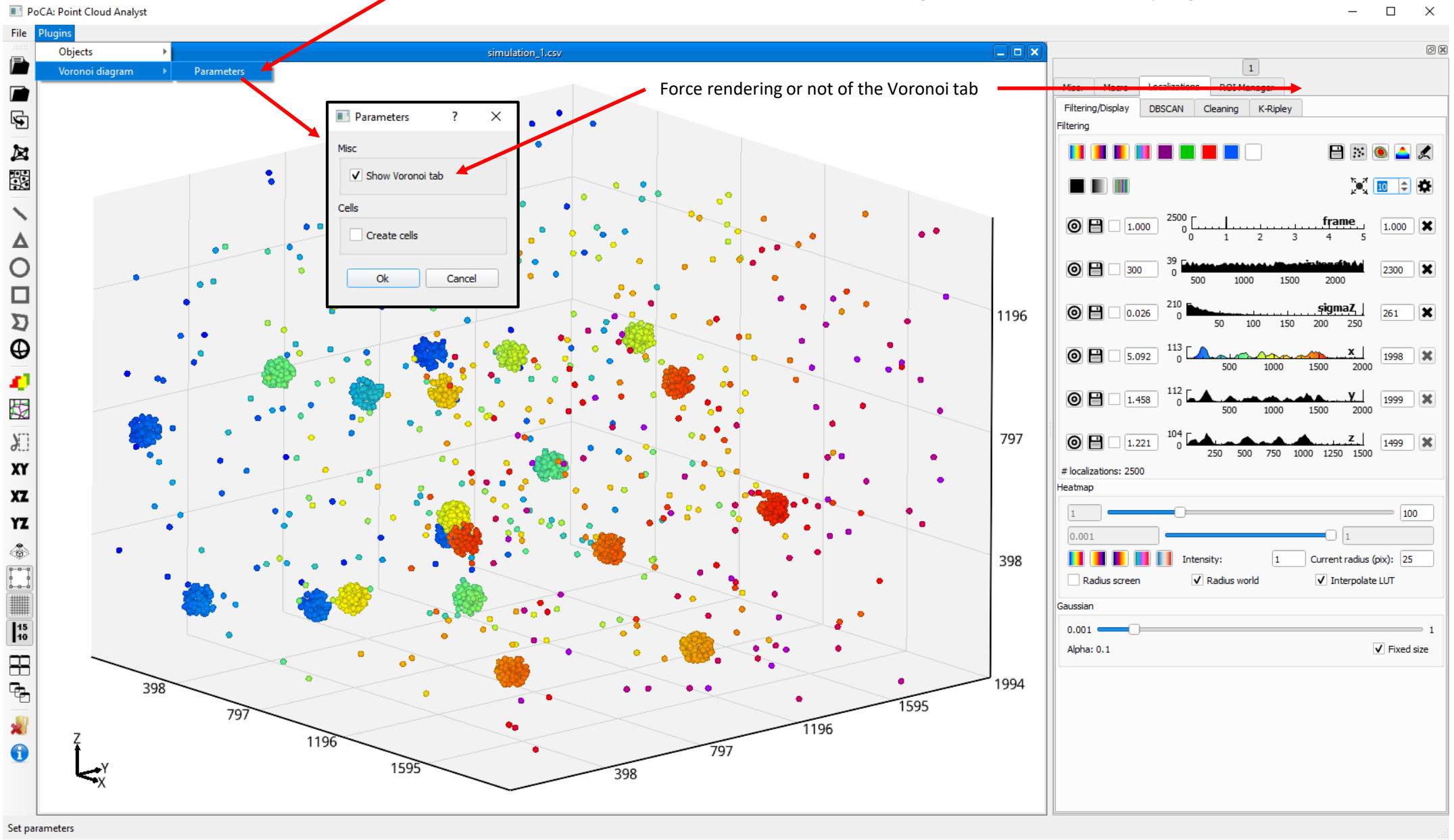


Delaunay triangulation

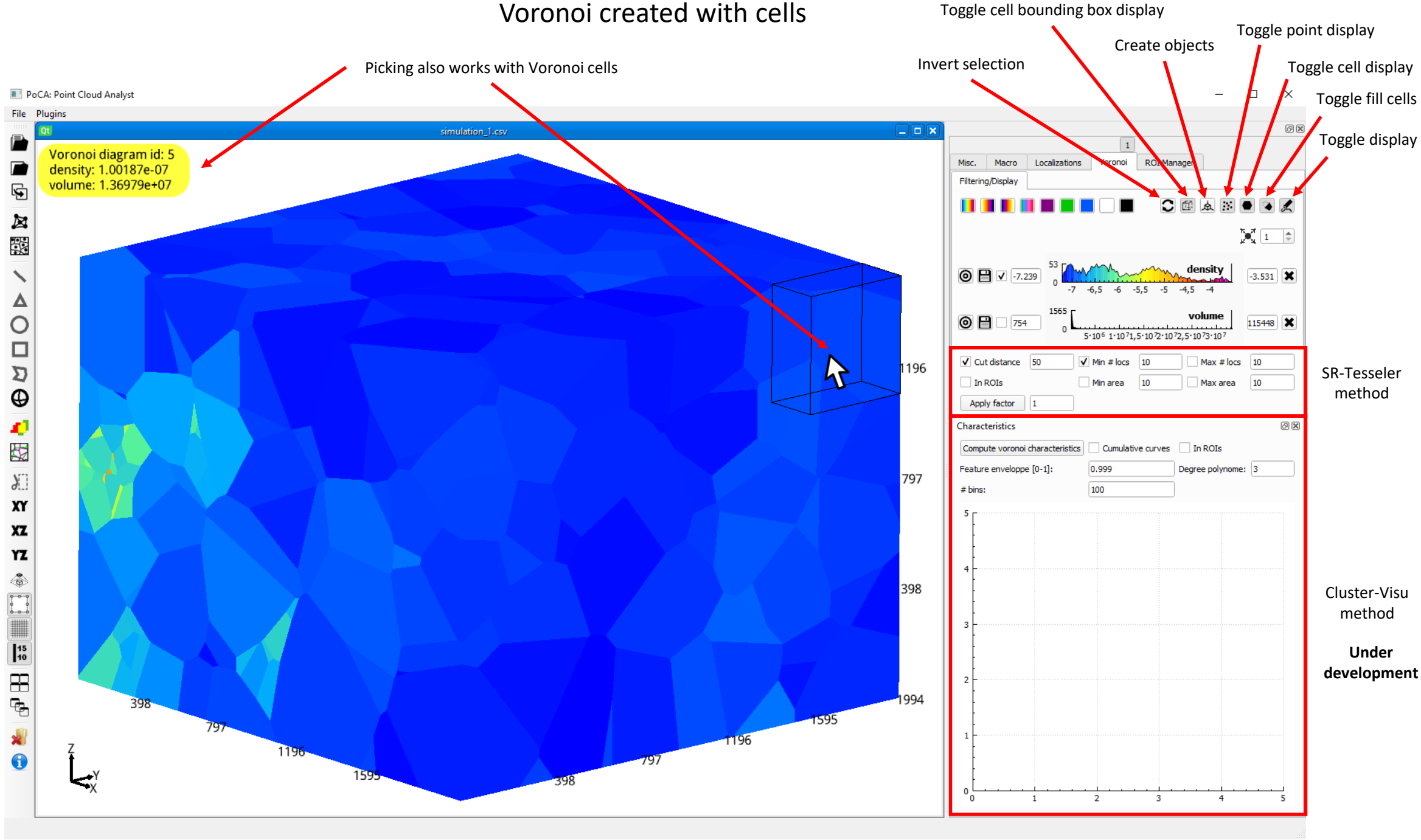


Voronoi creation

Voronoi can be created with or without Voronoi cells. As retrieving the cells from the GPU is very long, Voronoi should be created without cells.



Voronoi created with cells

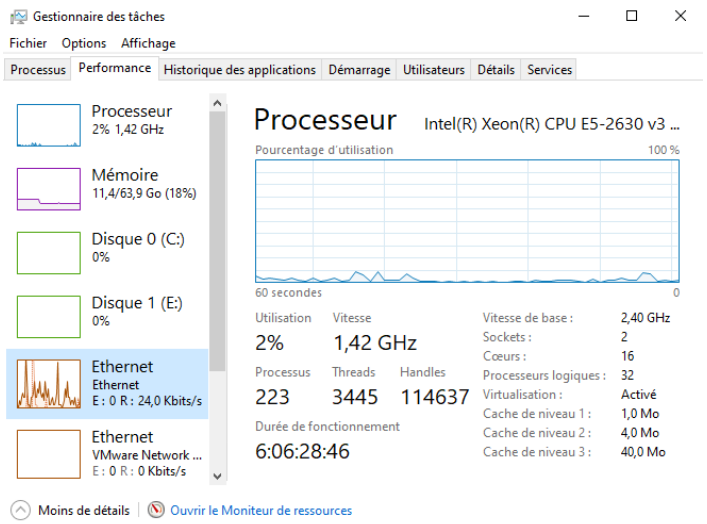


Voronoi timing

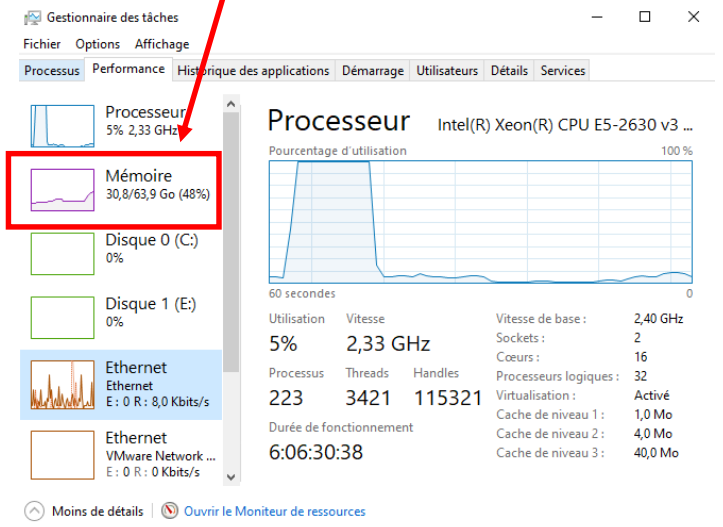
2D Voronoi on 6,000,000 localizations ≈ 40s
3D Voronoi on 4,000,000 localizations (without cells) ≈ 50s

3D Voronoi necessitates a Nvidia card and is very RAM demanding. If you hit 100% RAM usage, the software won't crash but the algorithm will freeze

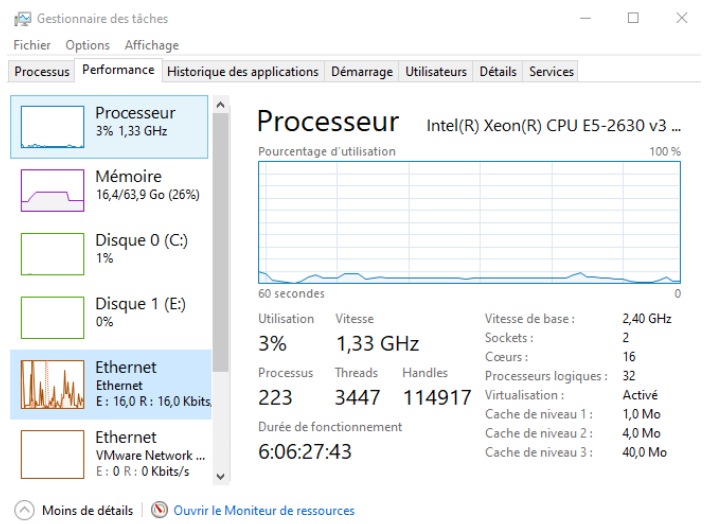
Baseline (before executing PoCA)



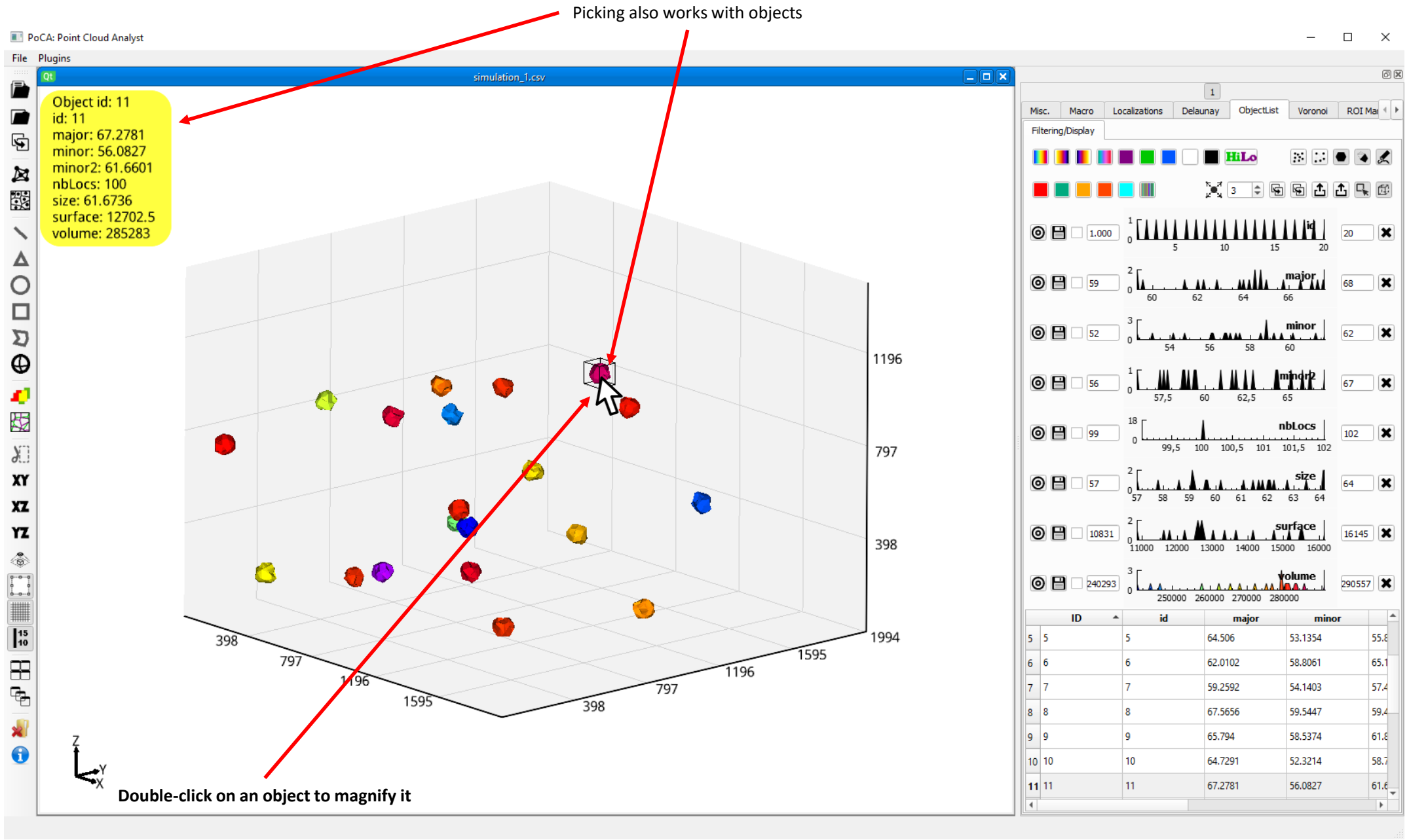
Memory peak during construction



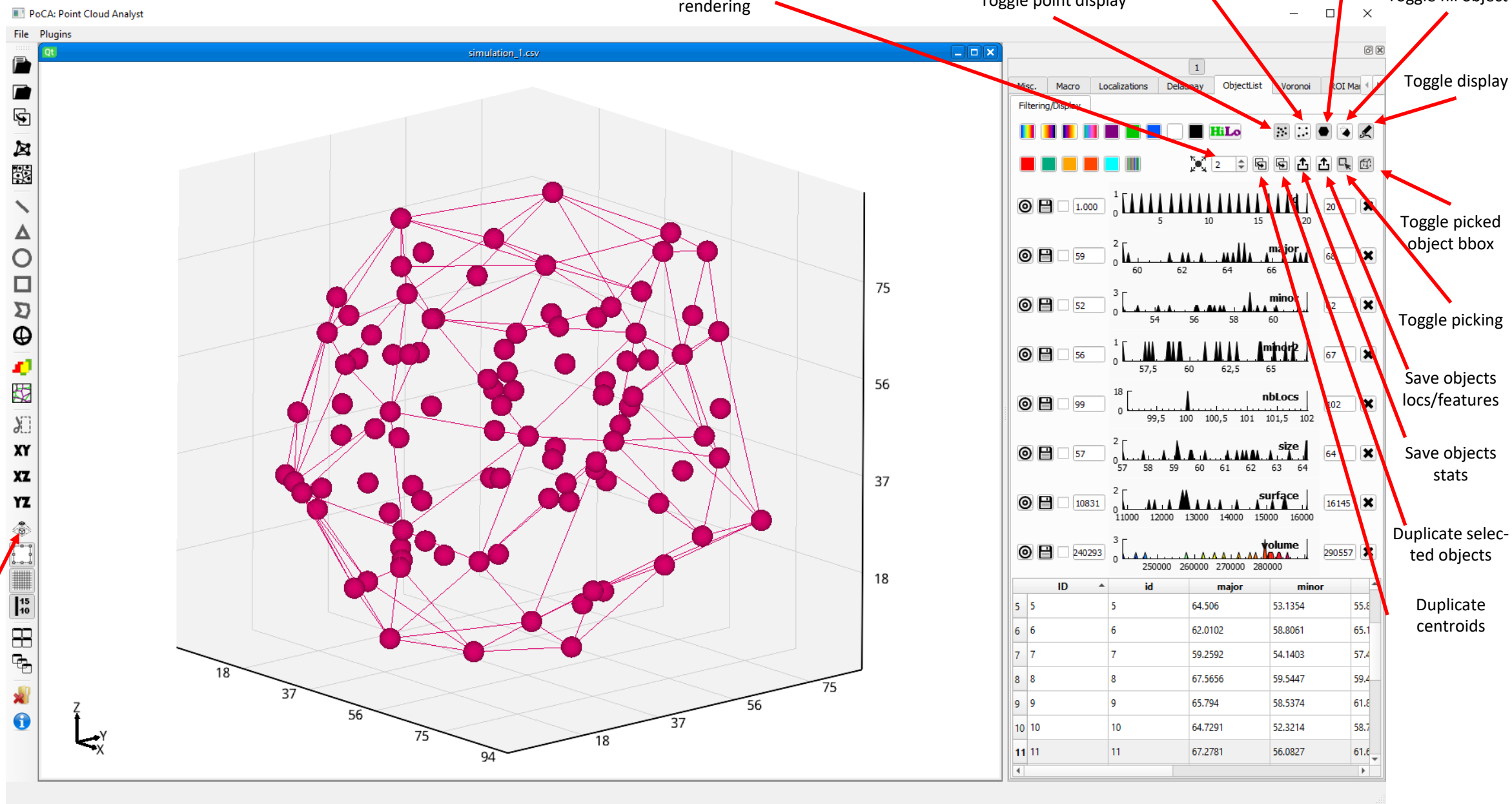
3D Voronoi on 4,000,000 locs done



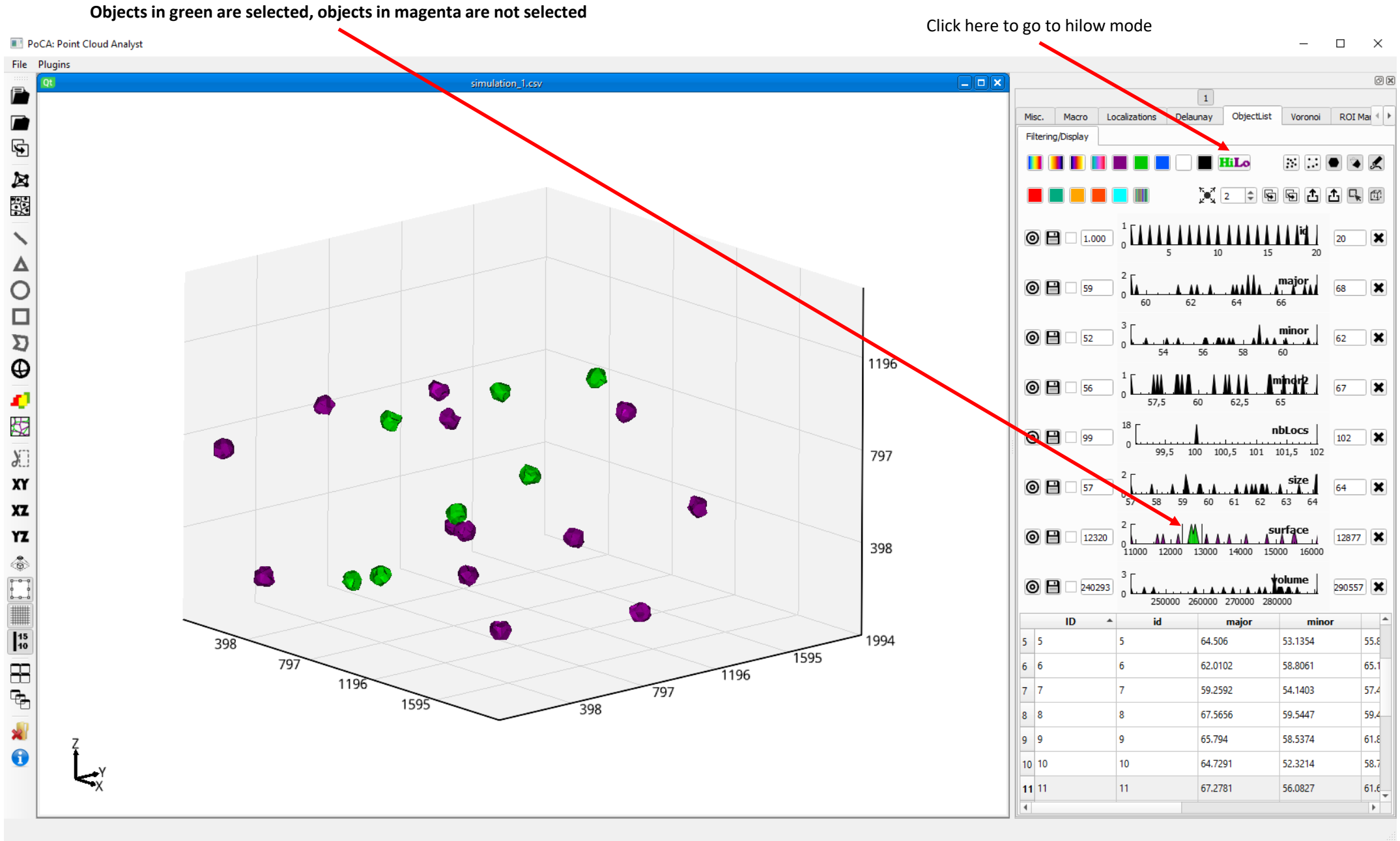
ObjectList tab



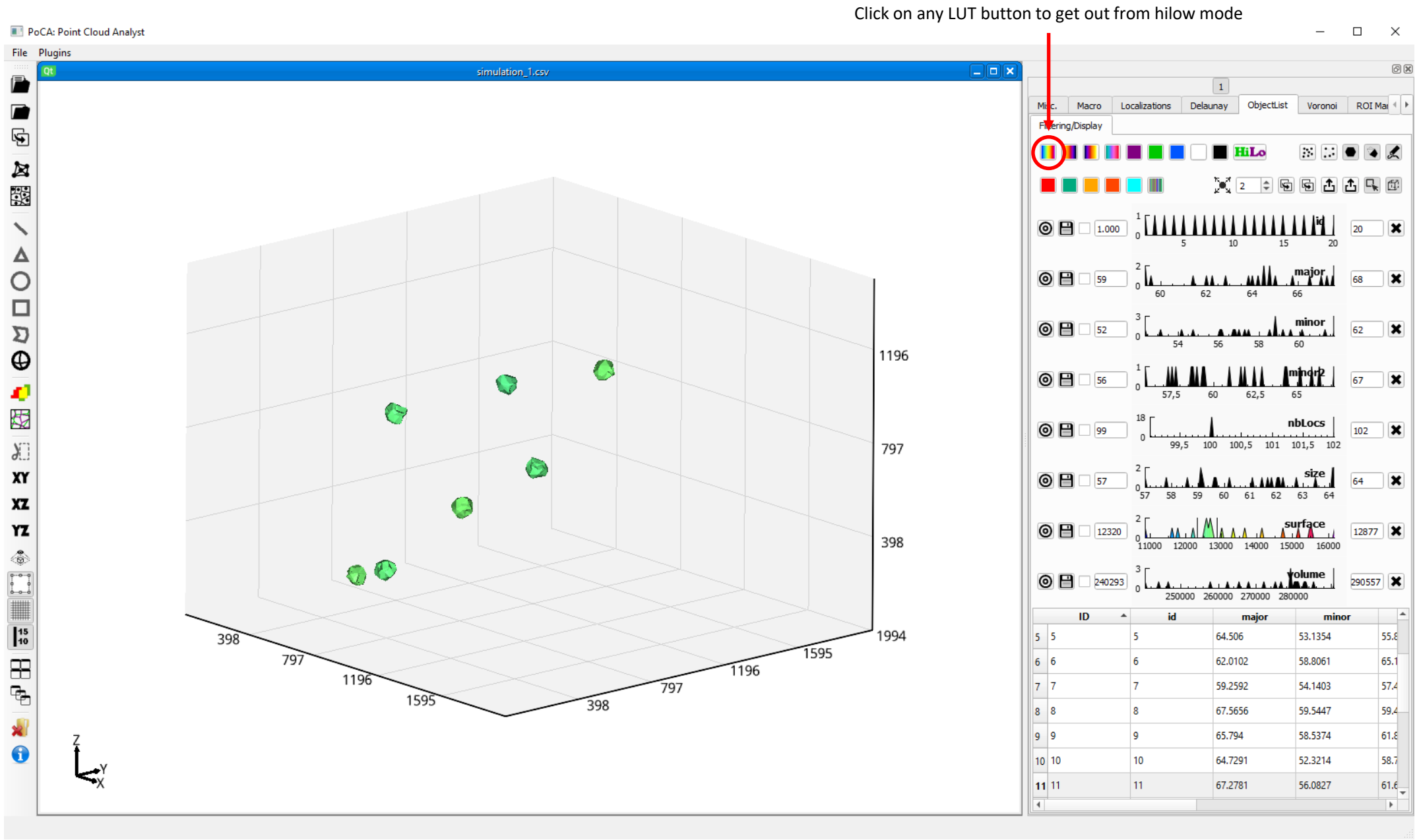
ObjectList tab



ObjectList tab: hilow display

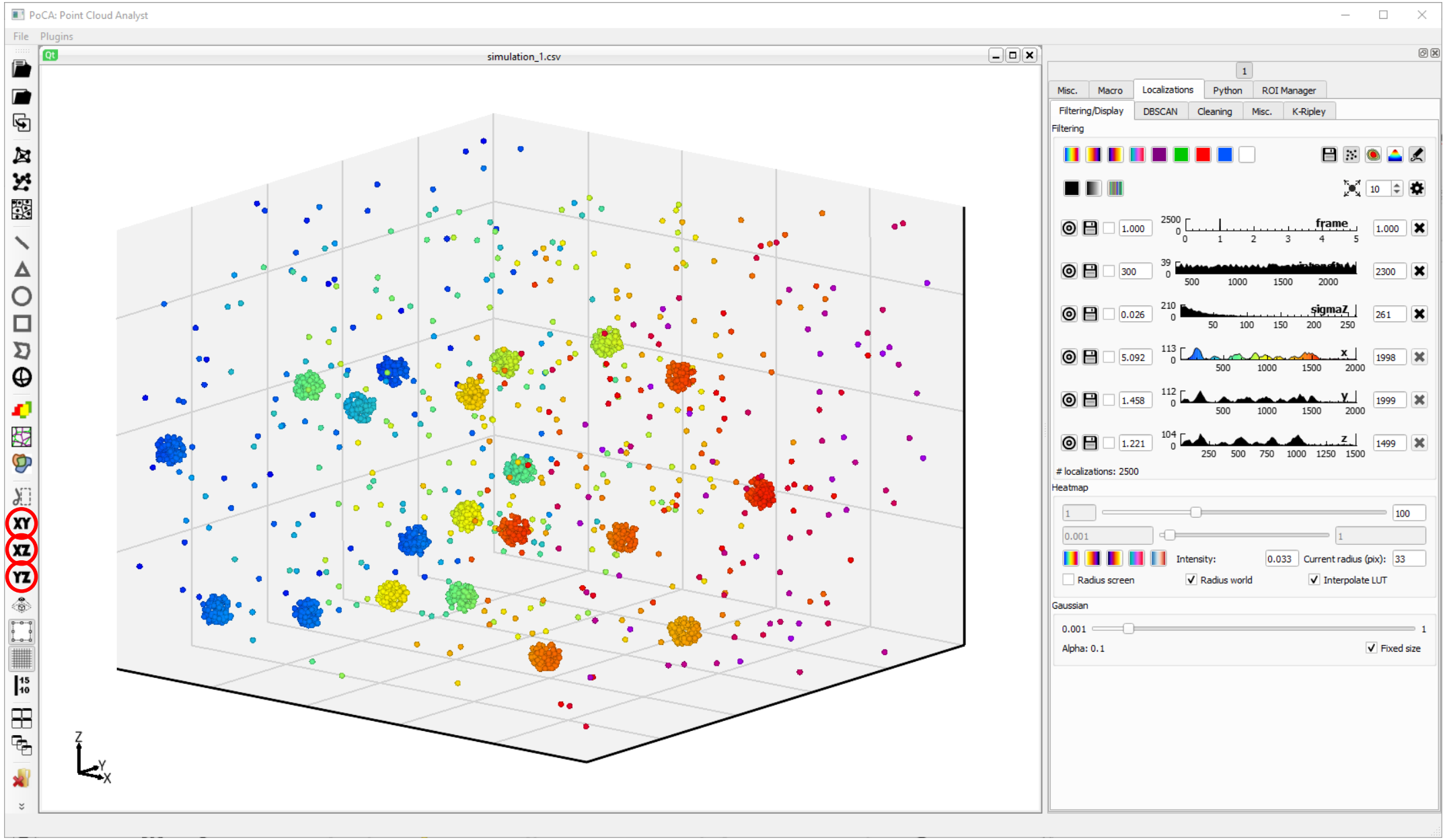


ObjectList tab: same display with normal LUT selected



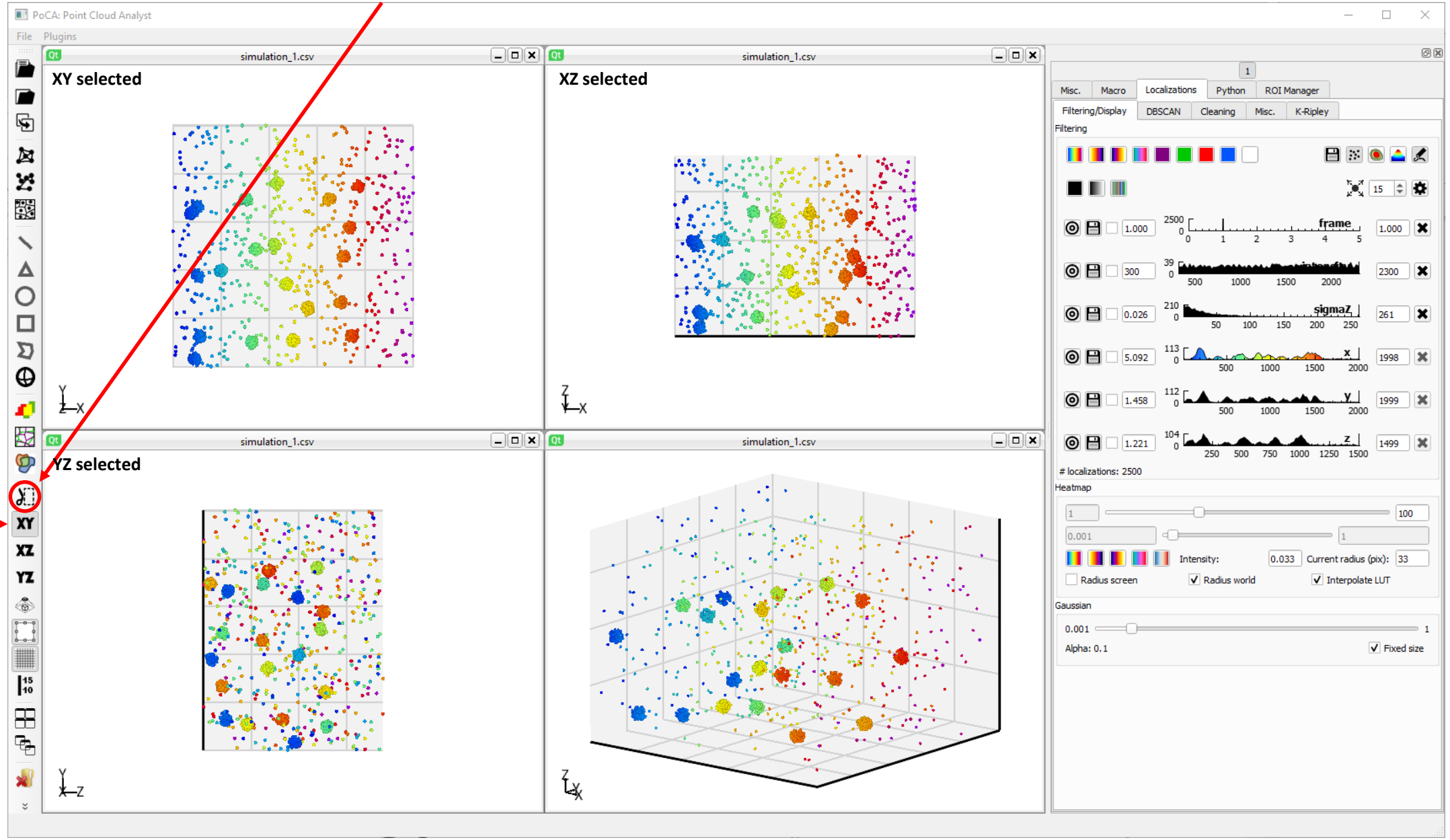
3D Viewer: crop

First, click on XY, XZ or YZ buttons to project the data to this frame



3D Viewer: crop

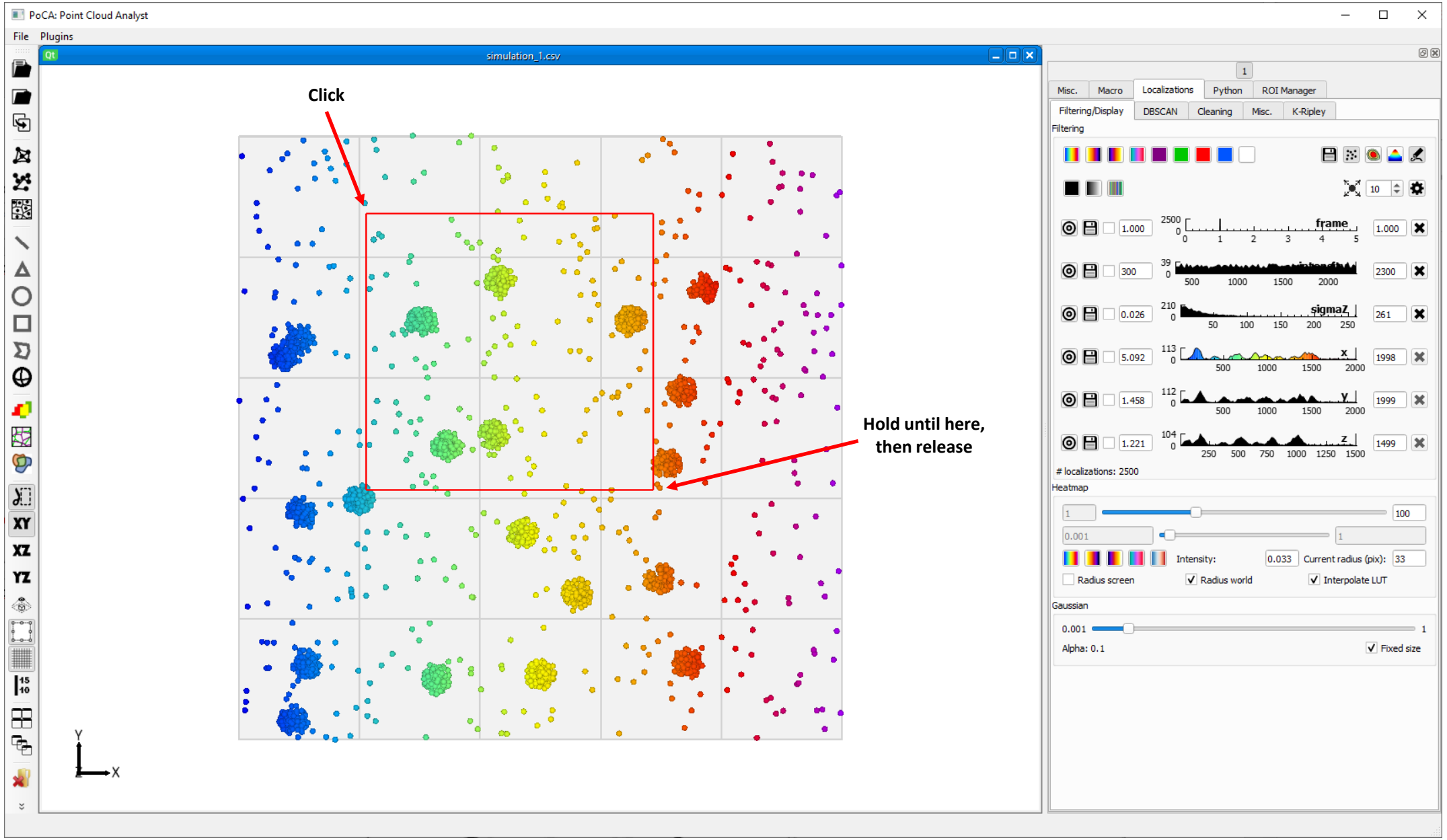
When one of these button is selected, the crop button becomes usable



XY was selected

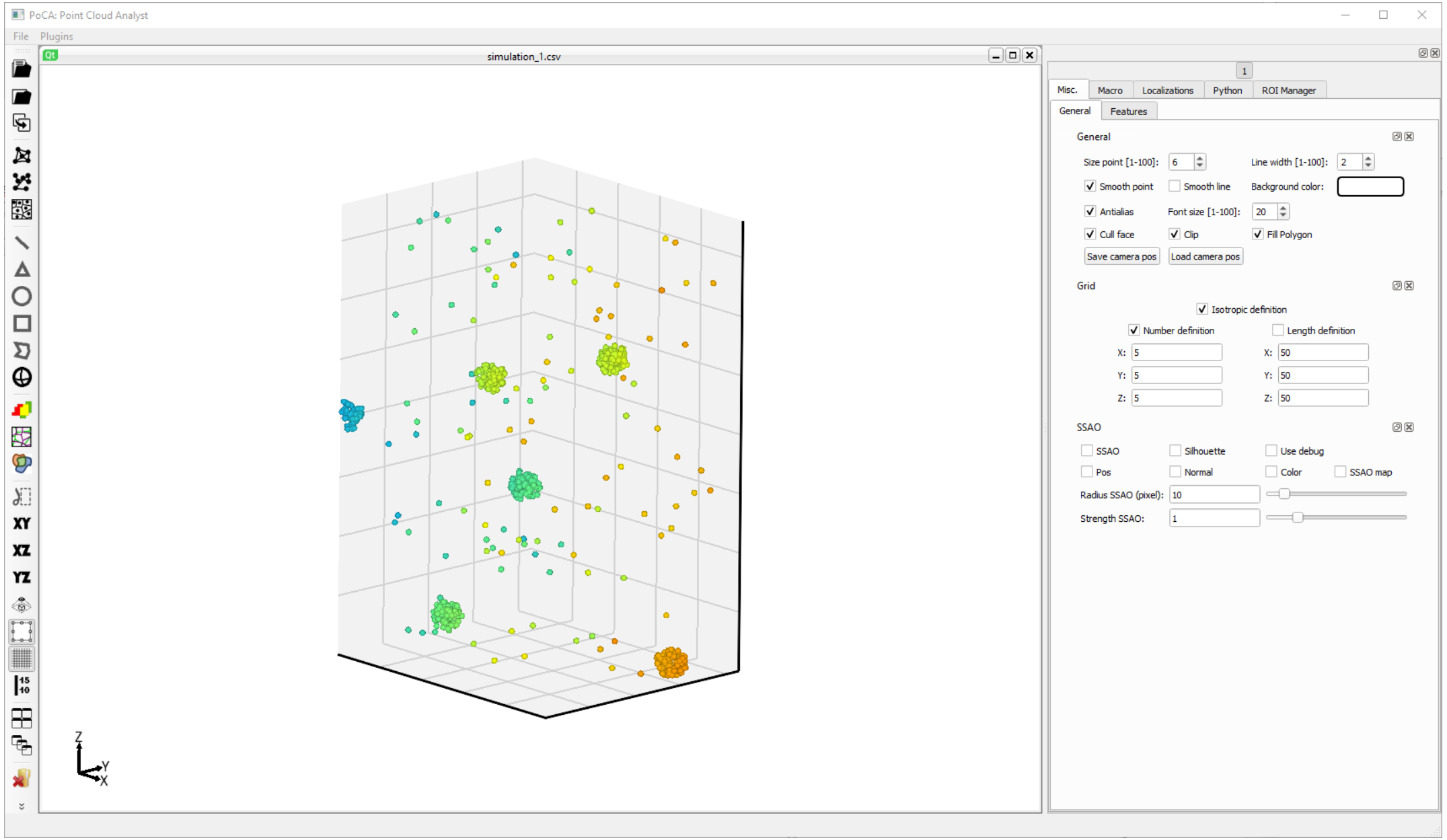
3D Viewer: crop

You can then click on the data, hold and move the mouse. When the button is released, the data will be cropped



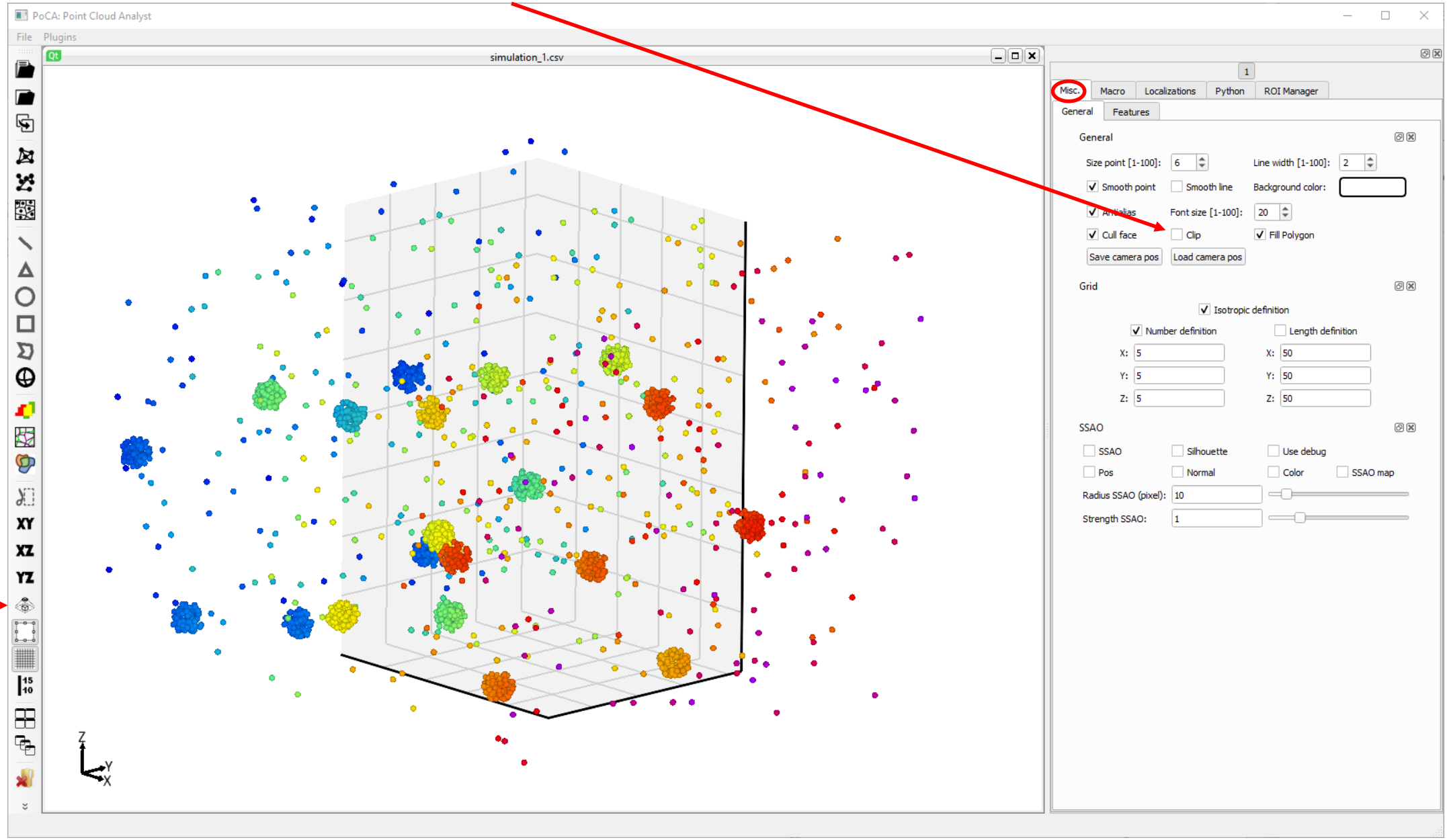
3D Viewer: crop

The [XY, XZ, YZ] button and the crop button have to be unselected to be able to rotate the data again



3D Viewer: crop

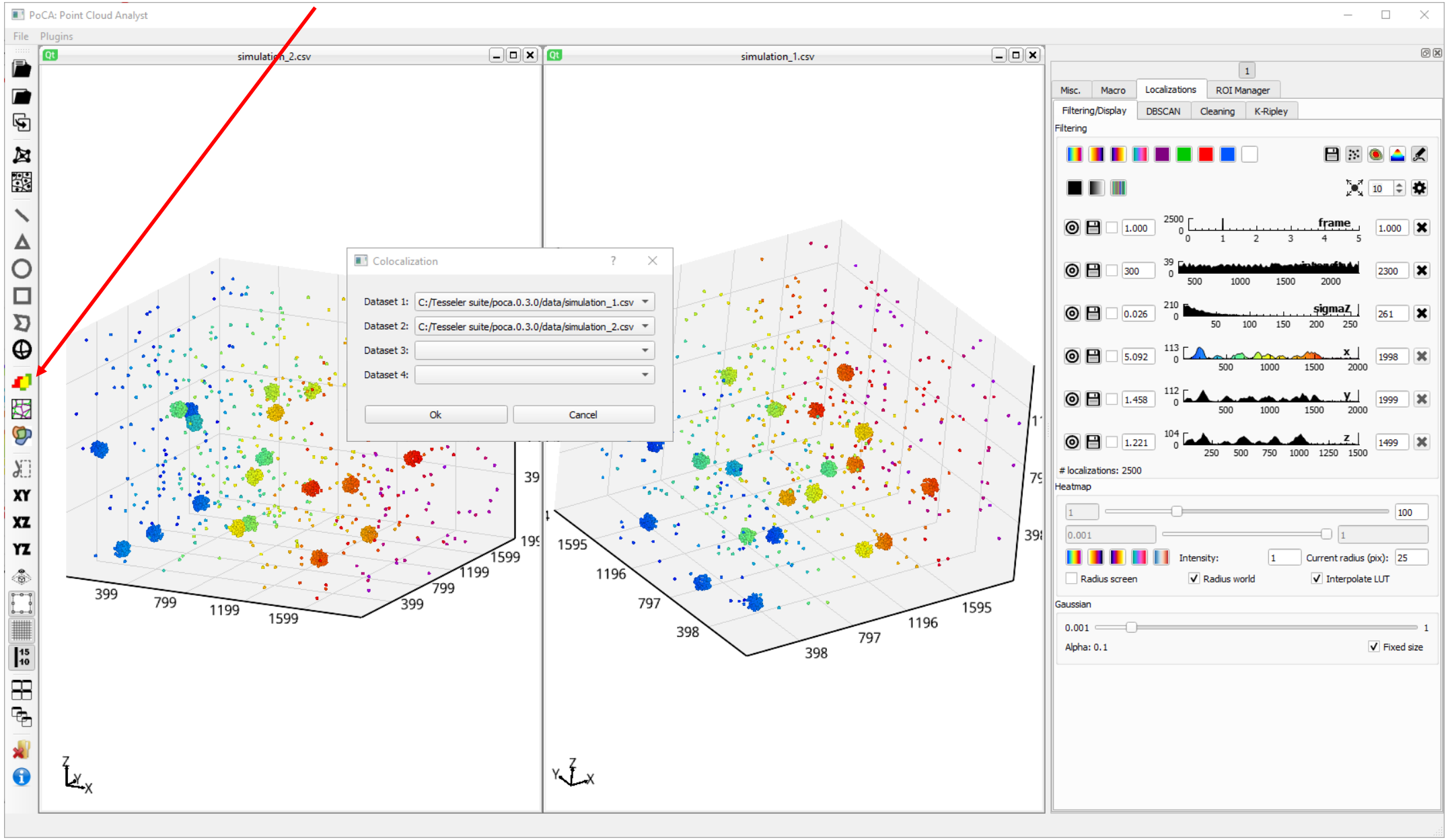
Crop is based on OpenGL clipping, the clip checkbox has to be checked for it to work



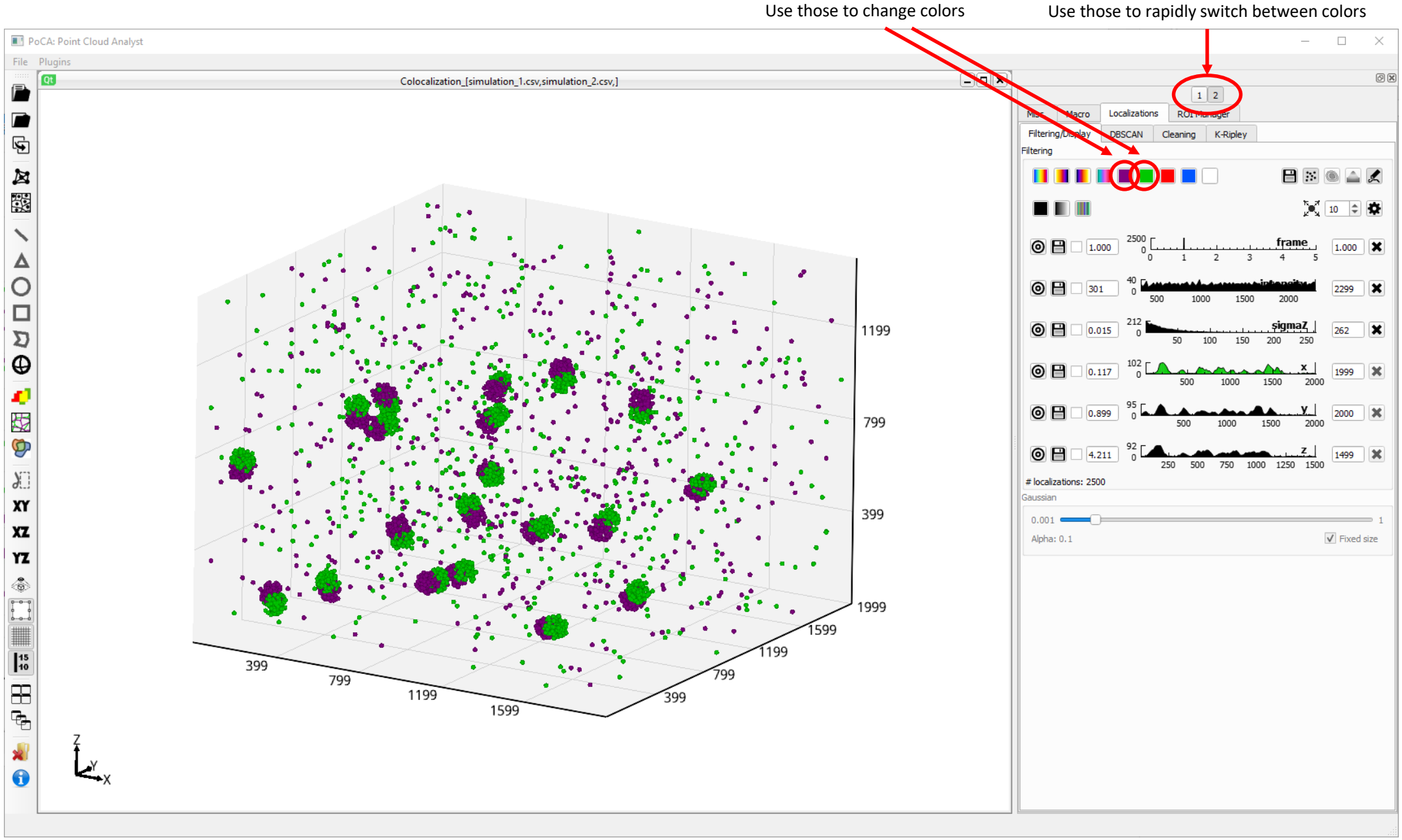
Click here to reset cropping

Colocalization object

Open 2 datasets and click this button



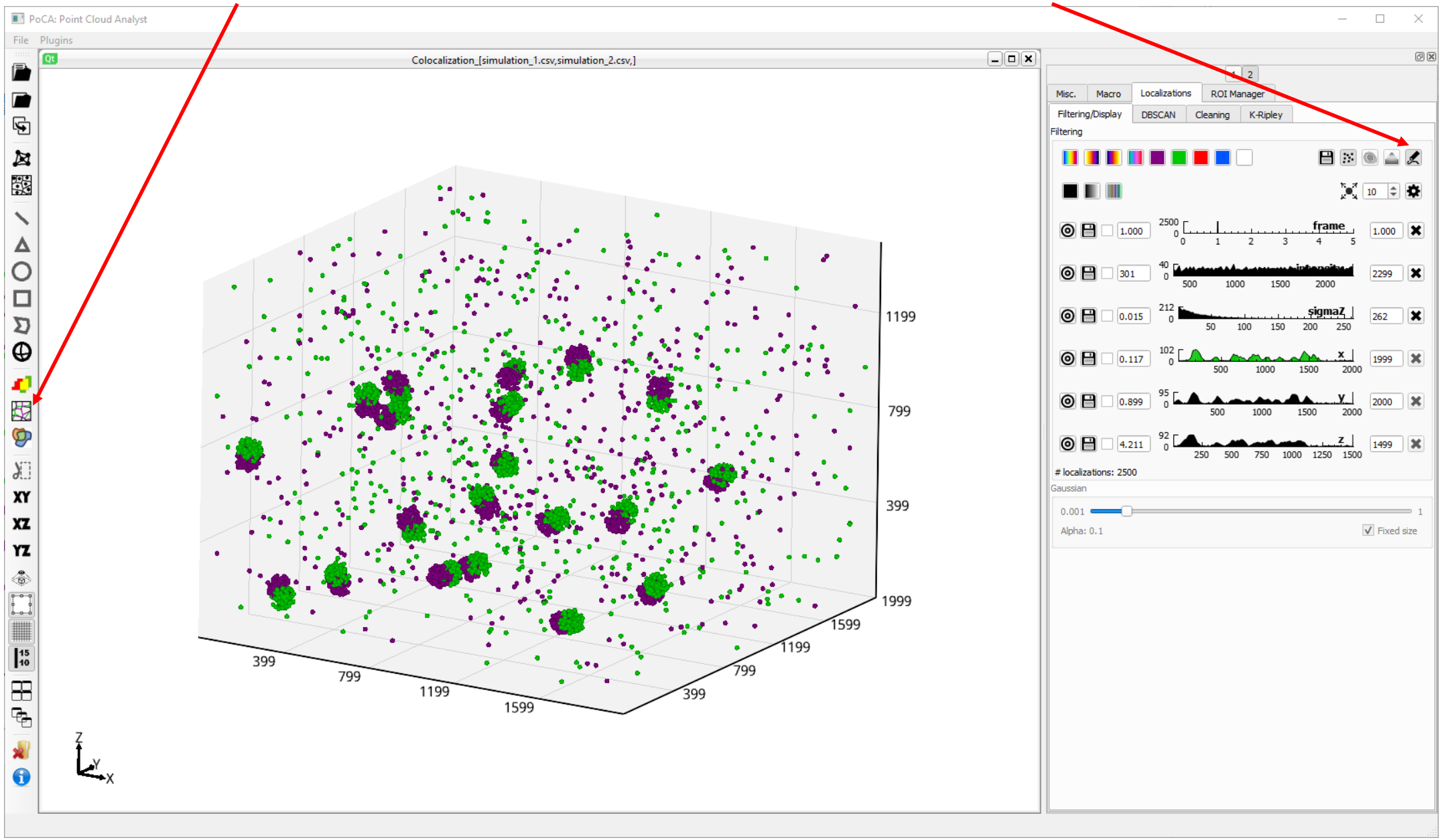
Colocalization object



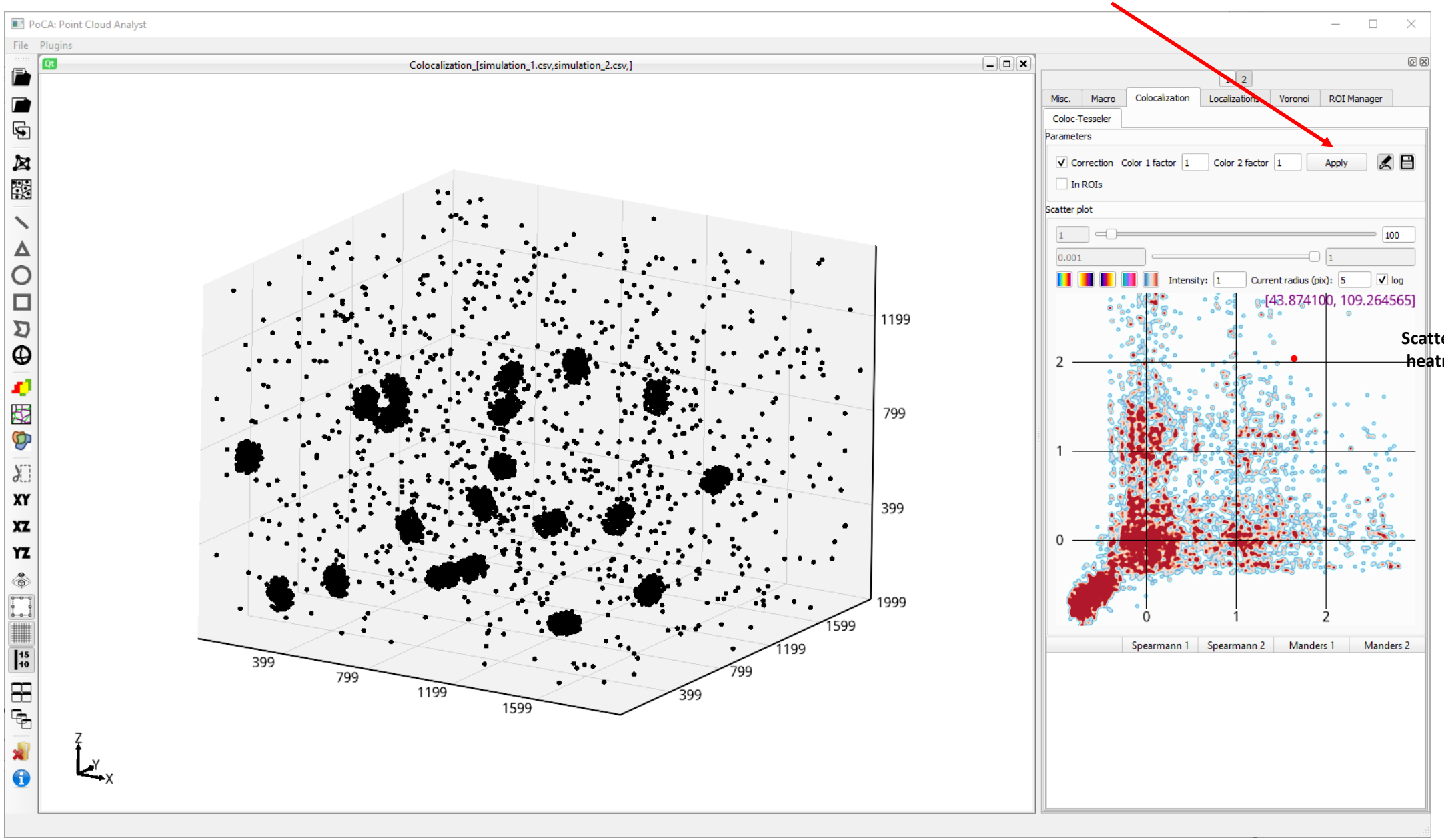
Coloc-Tesseler

Click here

Coloc-Tesseler displays is under the localization rendering, you need to switch off for both colors

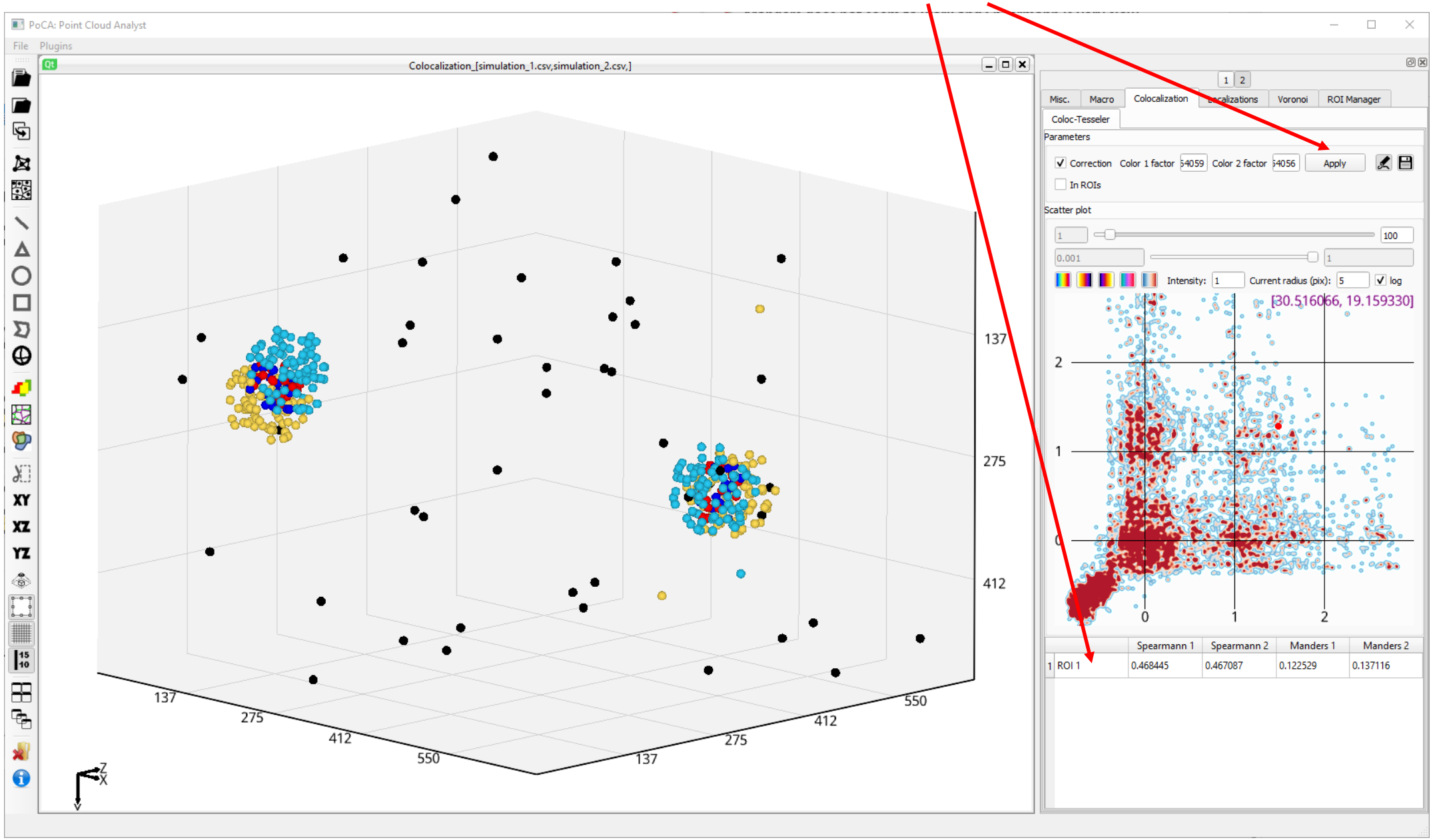


Coloc-Tesseler



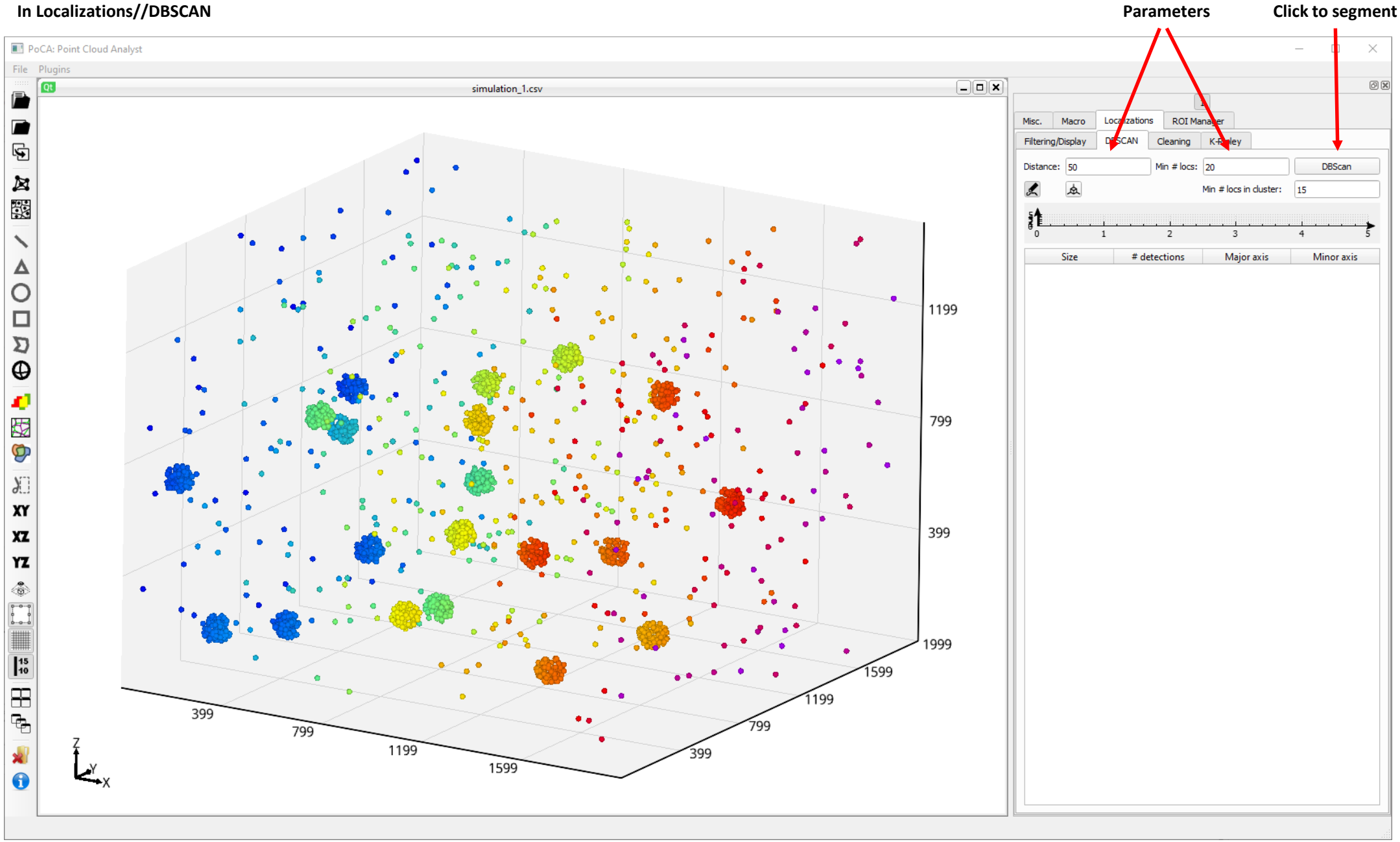
Coloc-Tesseler

Coloc-Tesseler is applied after setting the thresholds (textboxes or by cliking on the scatterplot) and clicking on « Apply »



DBSCAN

In Localizations//DBSCAN

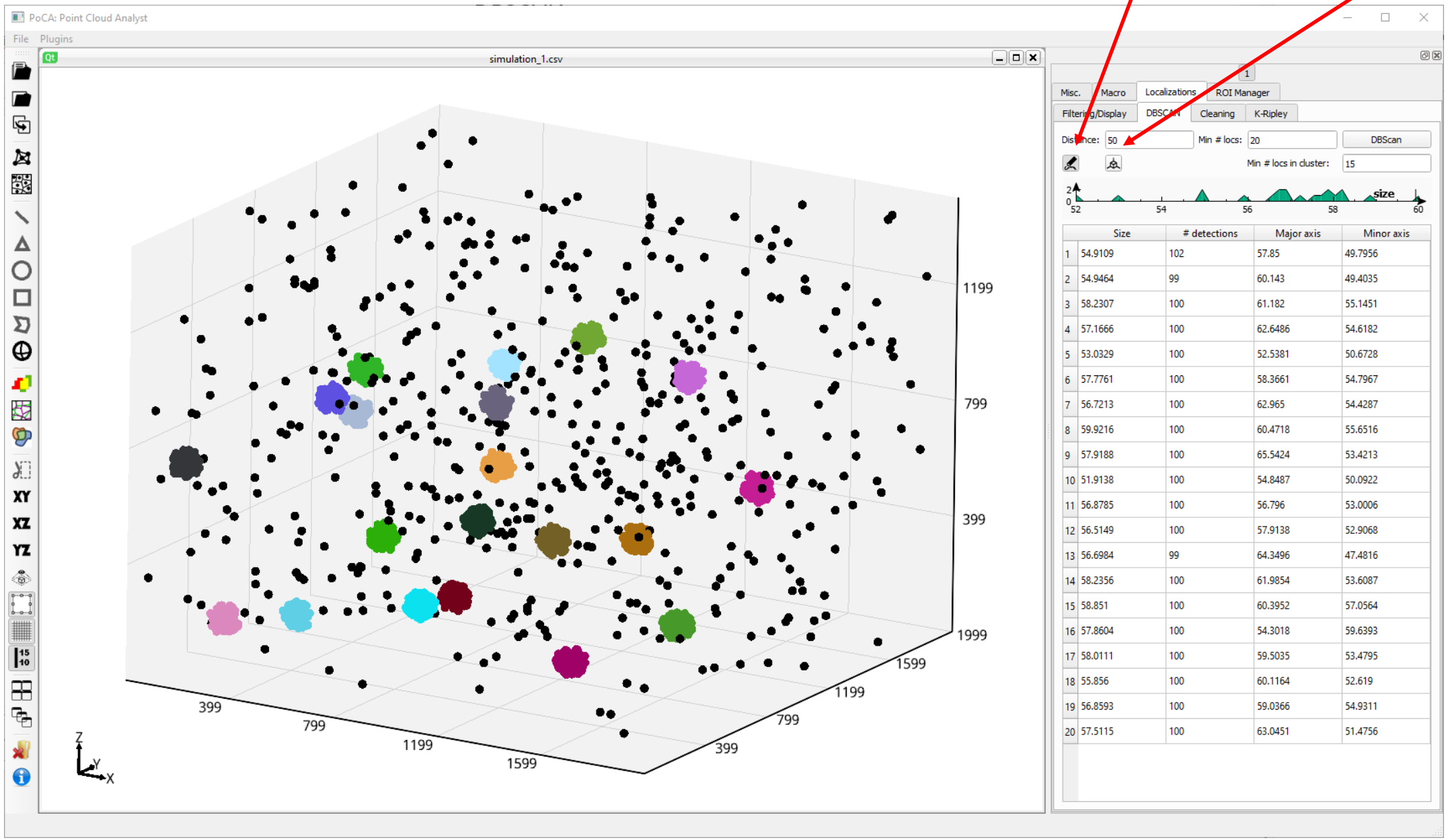


DBSCAN

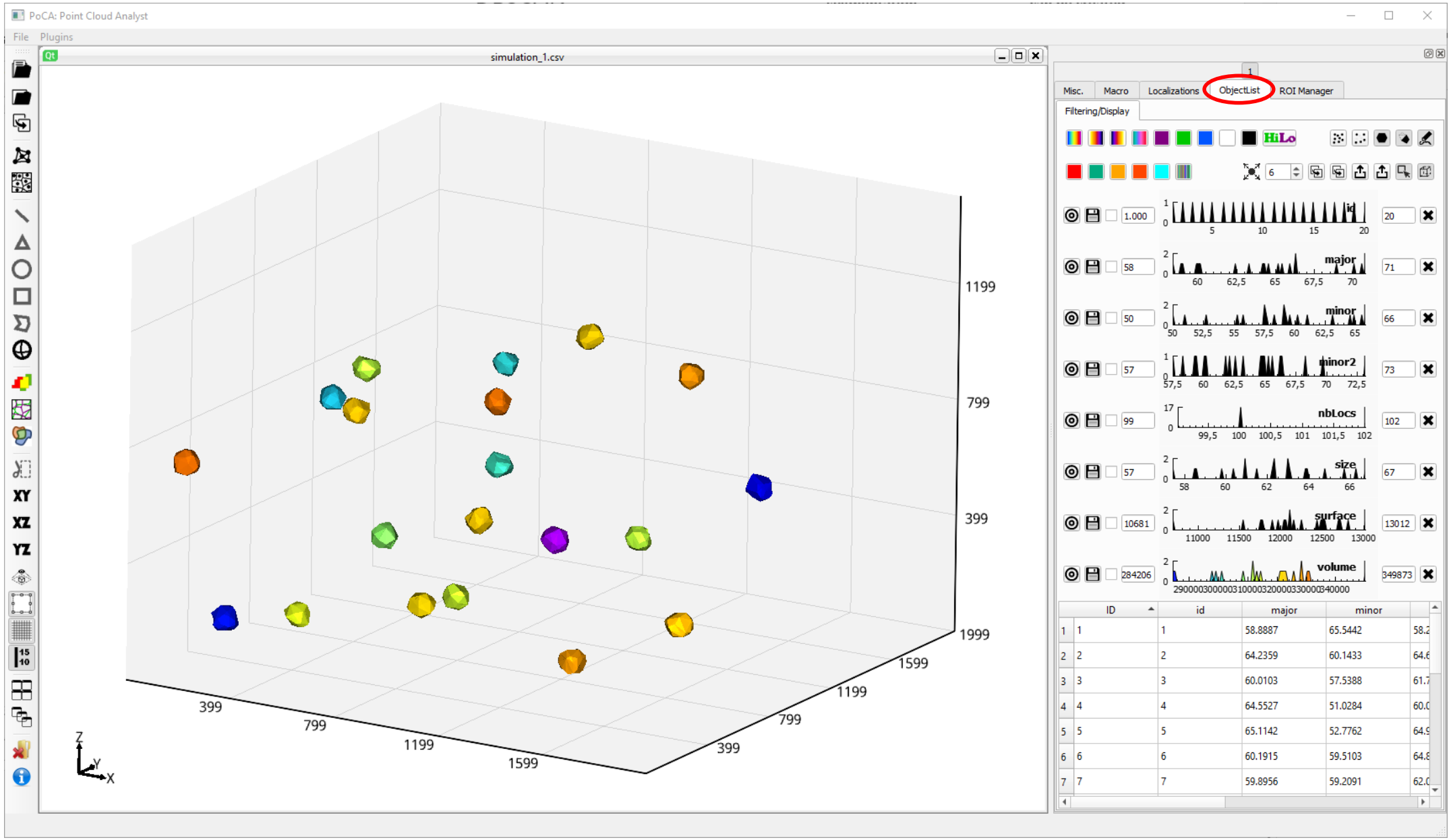
Switch DBSCAN segmentation display

PoCA's object can be created with this button

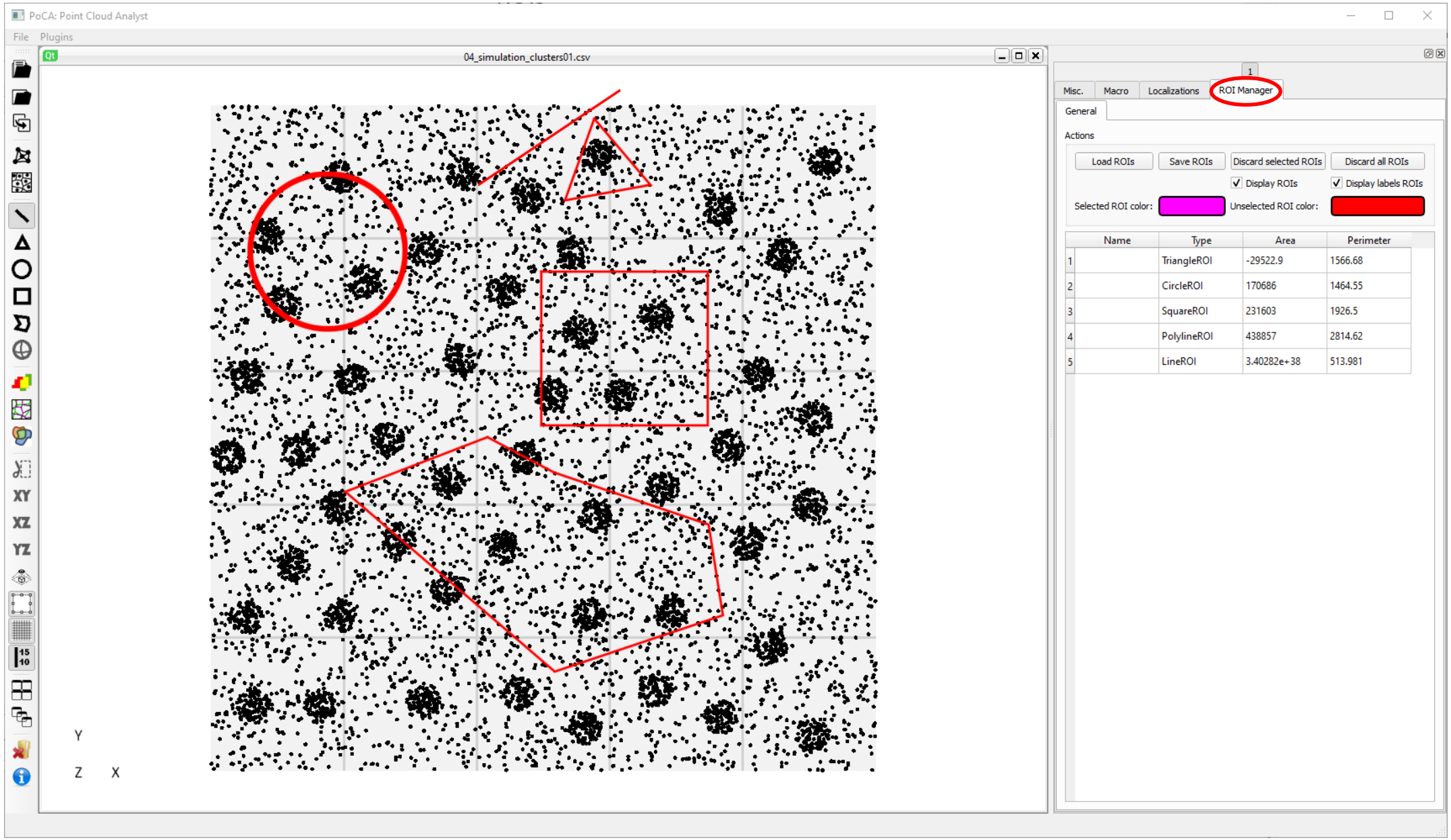
DBSCAN displays is under the localization rendering, you need to switch off this one before (in Localizations\\Filtering/Display)



DBSCAN


PoCA: Point Cloud Analyst
File
Plugins
simulation_1.csv
Misc.
Macro
Localizations
ObjectList
ROI Manager
Filtering/Display
HiLo
id
major
minor
minor2
nbLocs
size
surface
volume
ID
id
major
minor
XY
XZ
YZ
399
799
1199
1599
1999
Z
Y
X

ROIs – 2D



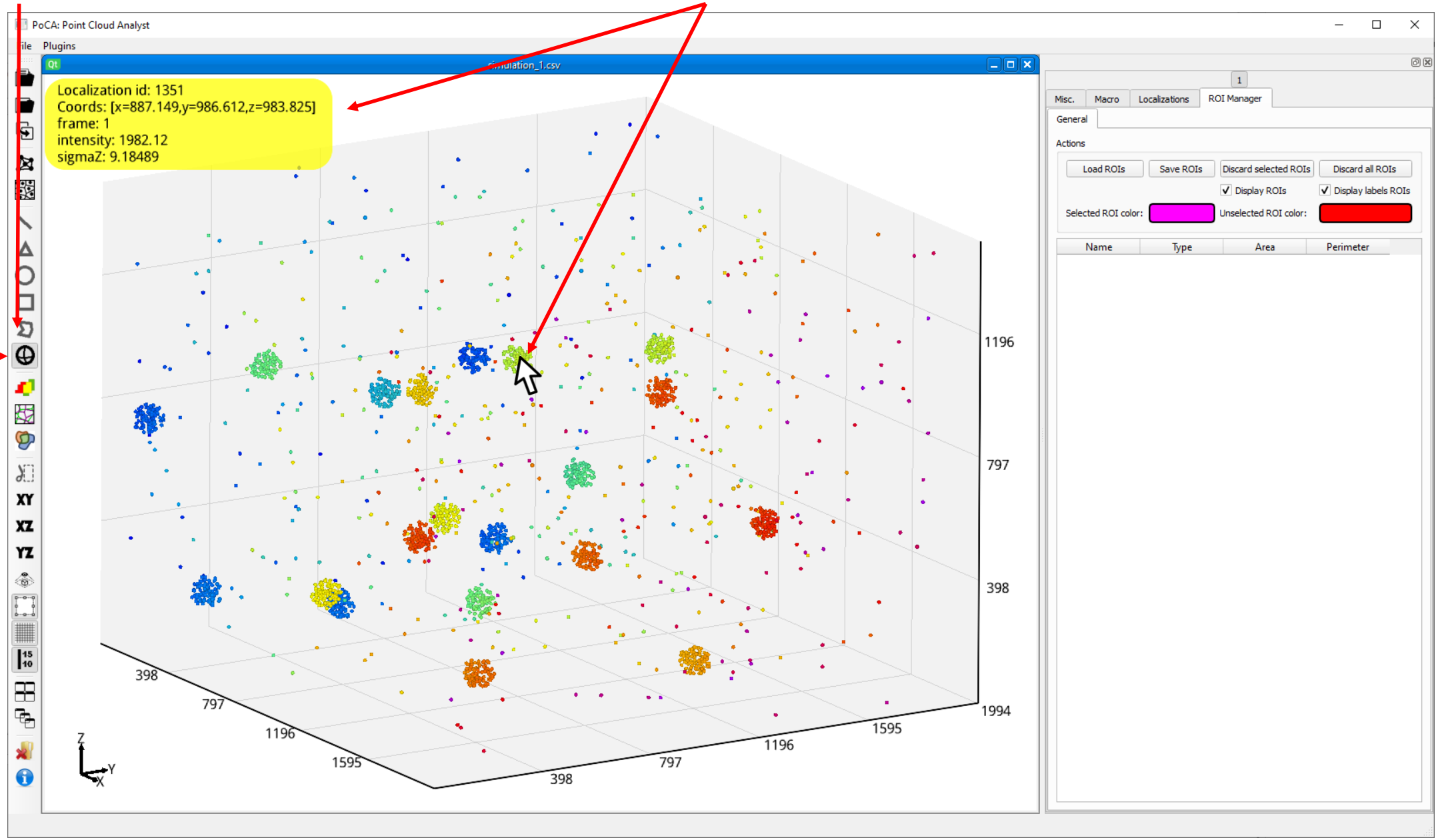
2D ROIs

ROIs – 3D

1. Select the sphere ROI button

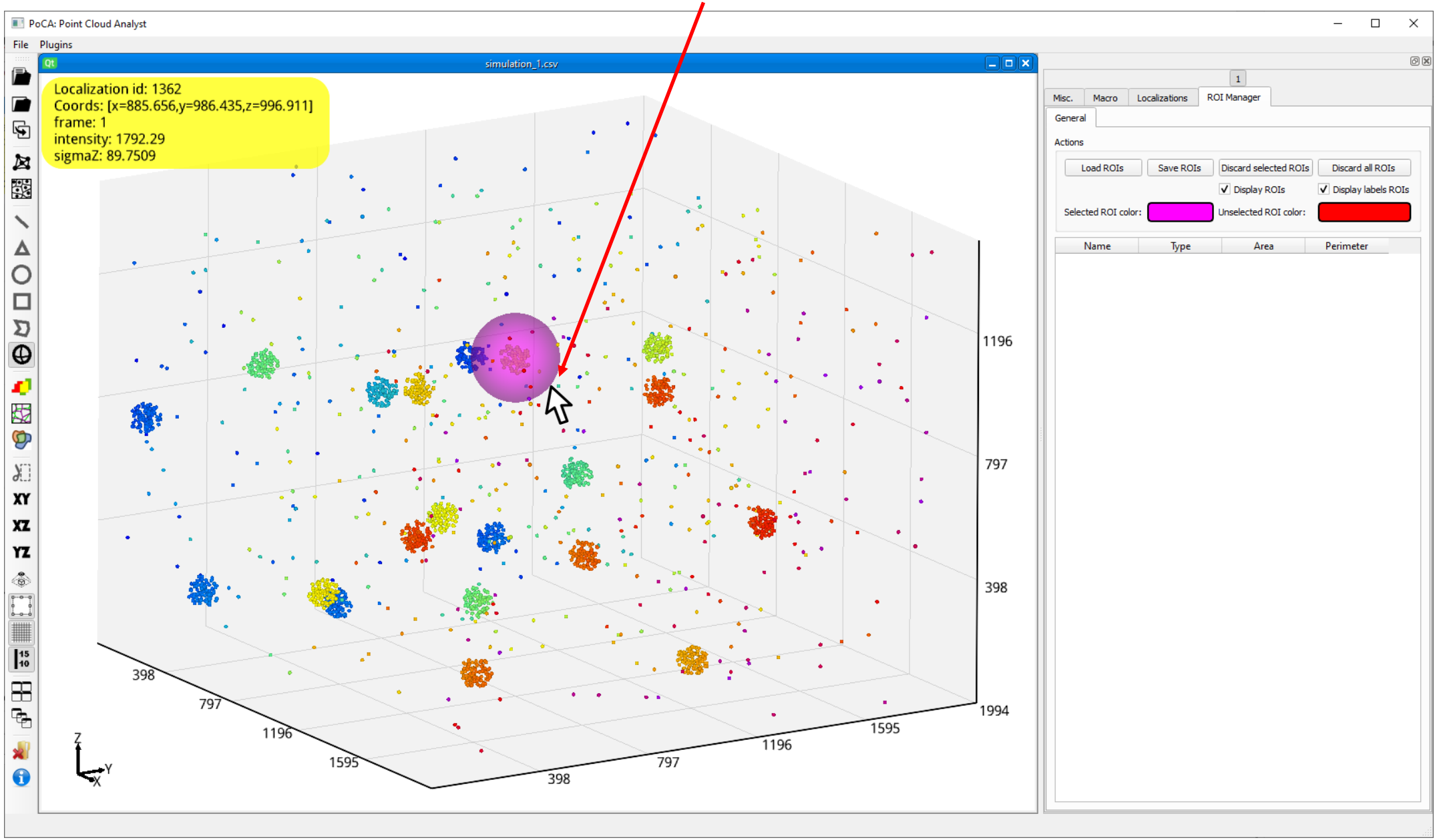
2. Pick a localization (required step because one pixel corresponds to a line in the 3D point of view)

3D ROIs



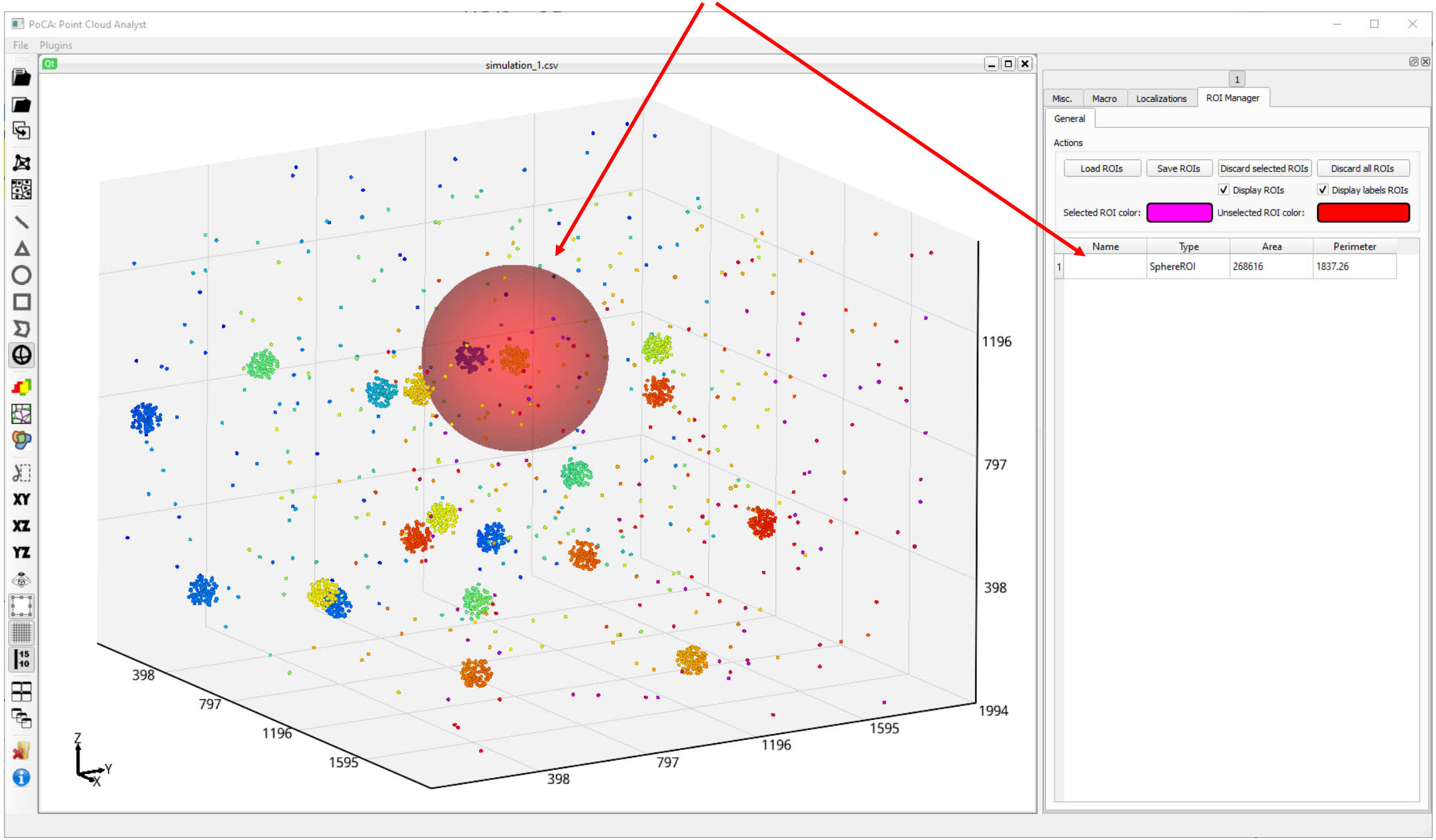
ROIs – 3D

3. Click and hold until the desired sphere ROI size



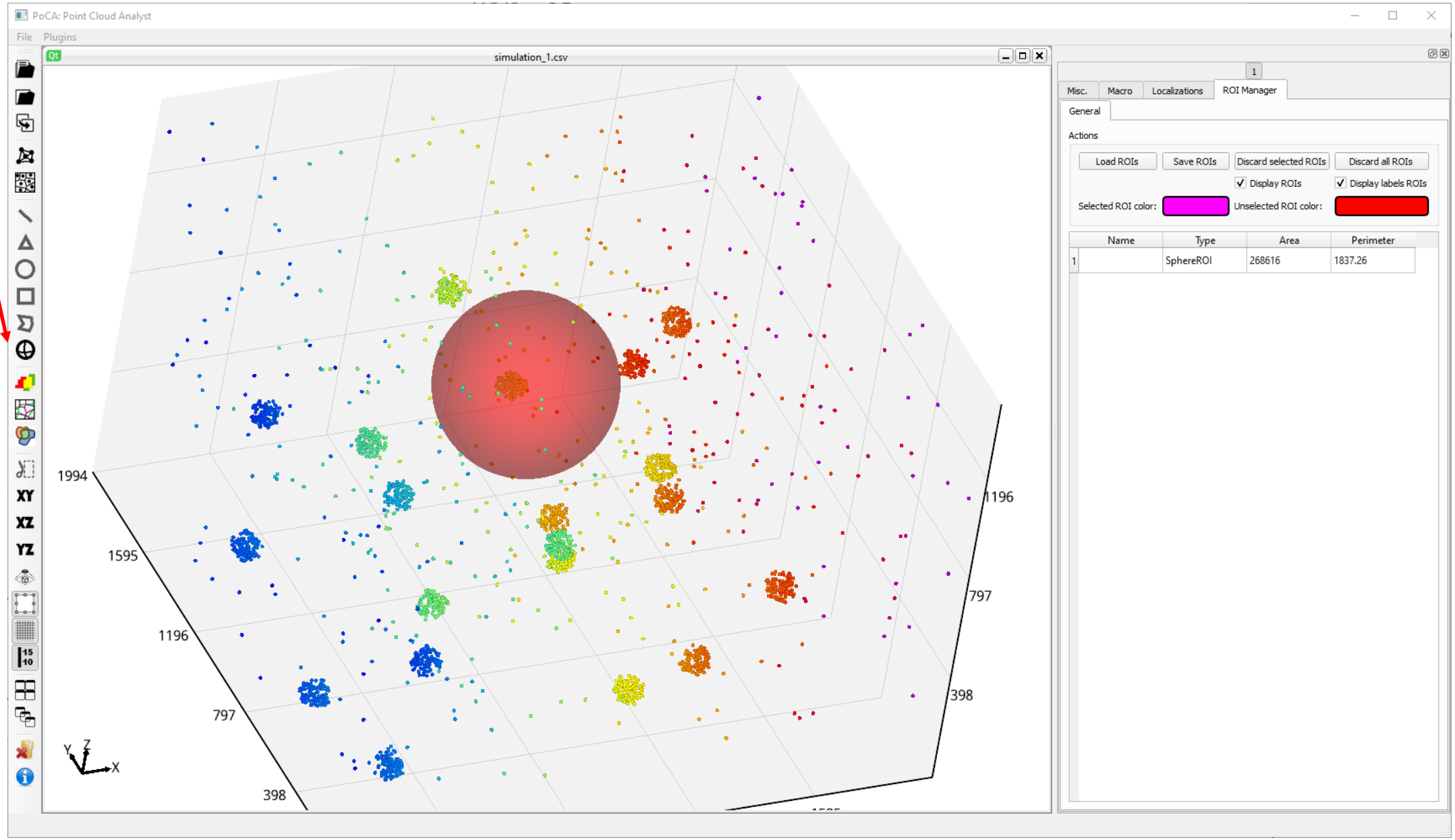
ROIs – 3D

4. Sphere ROI was created



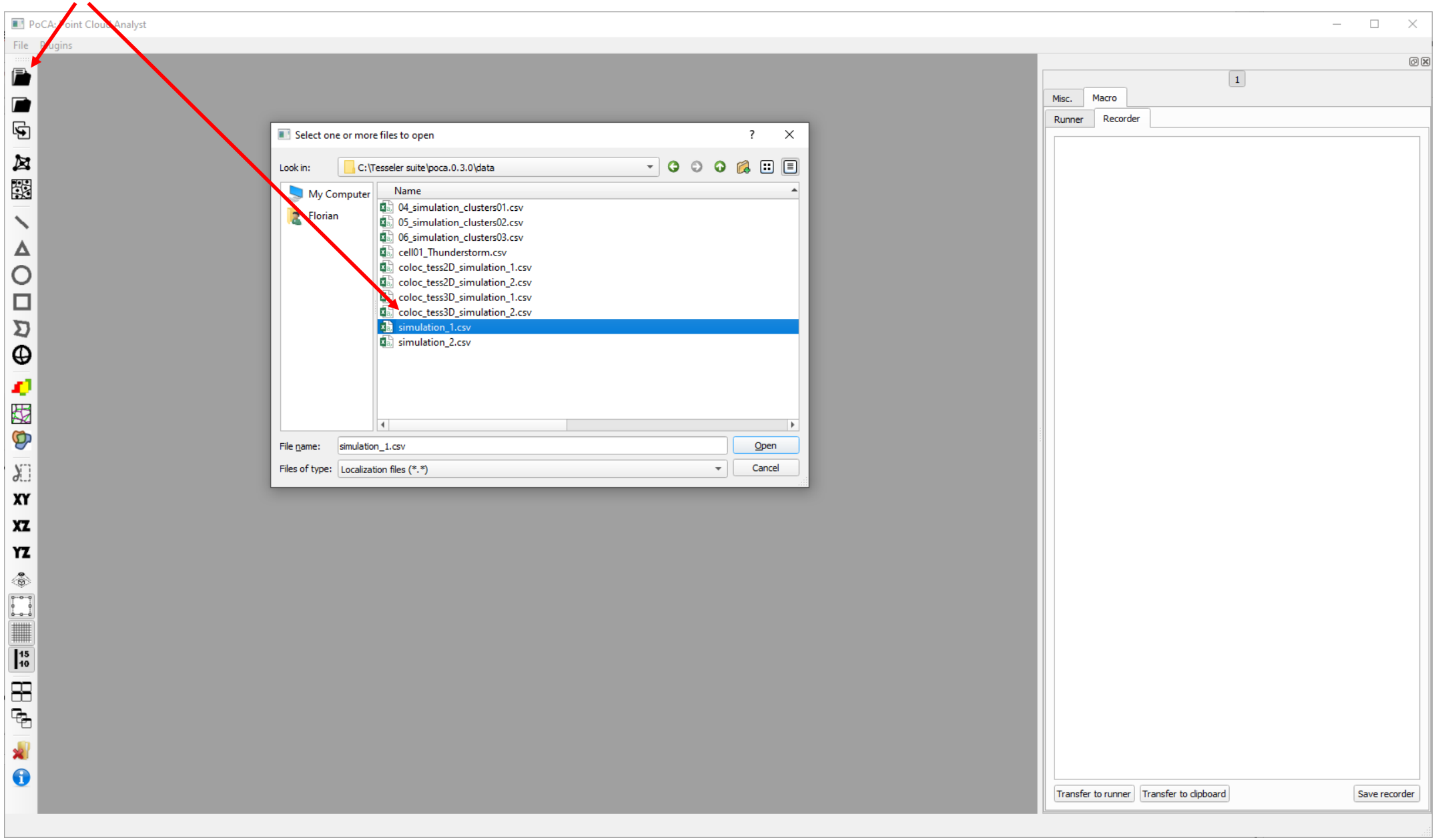
ROIs – 3D

Deselect to be able to rotate again



Macro

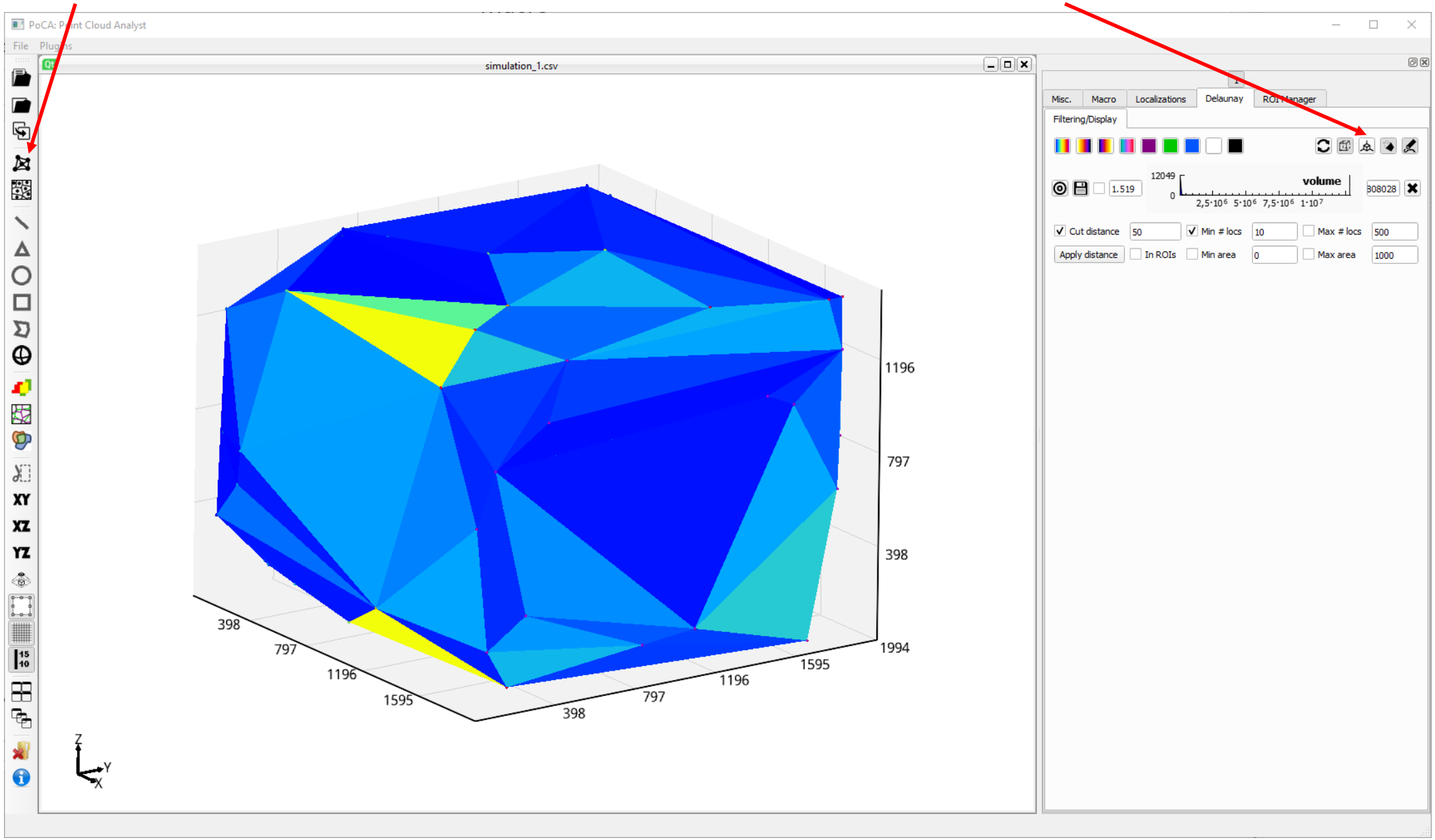
1. Open a dataset



Macro

2. Create a 3D Delaunay

3. Create objects

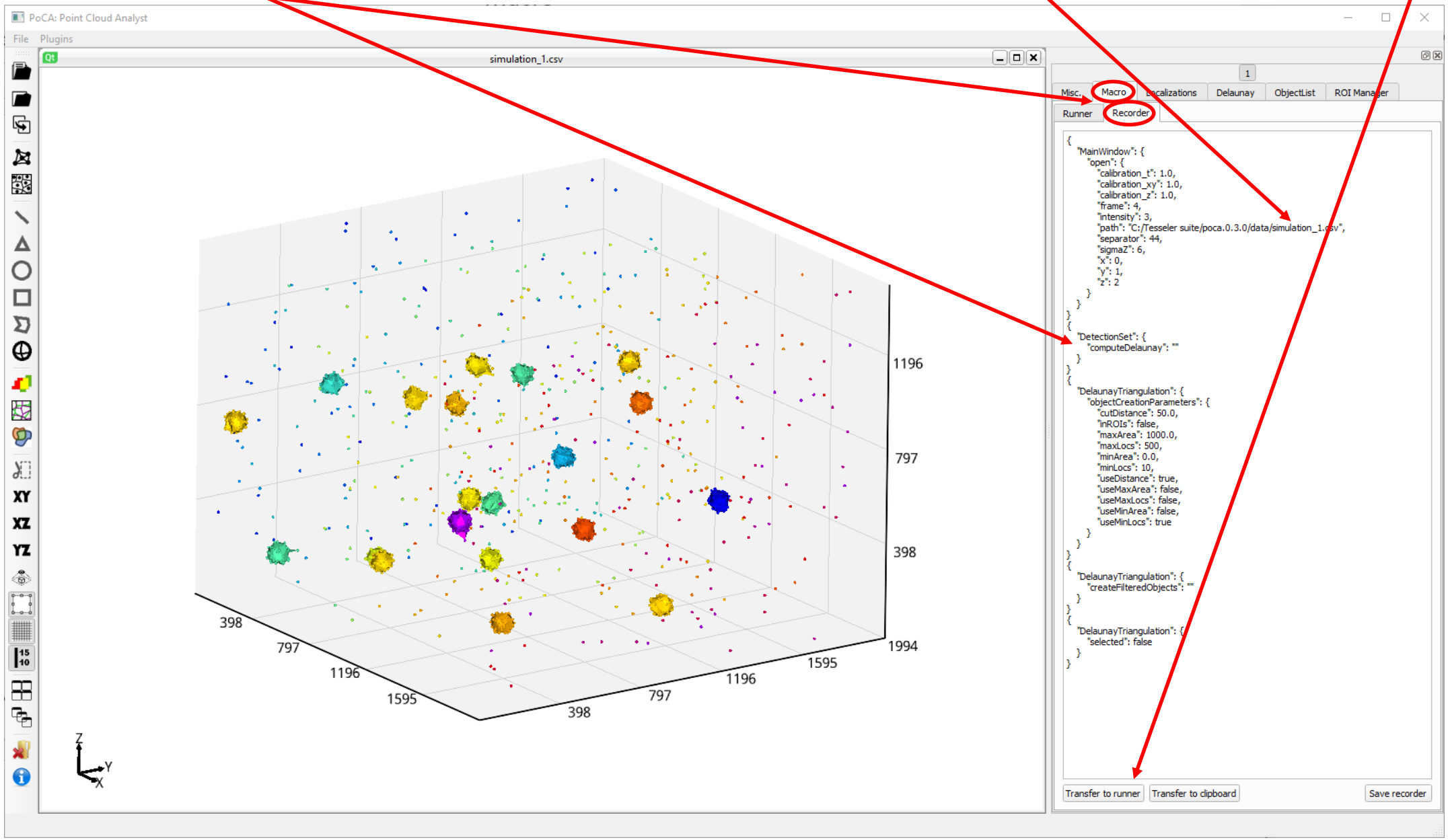


Macro

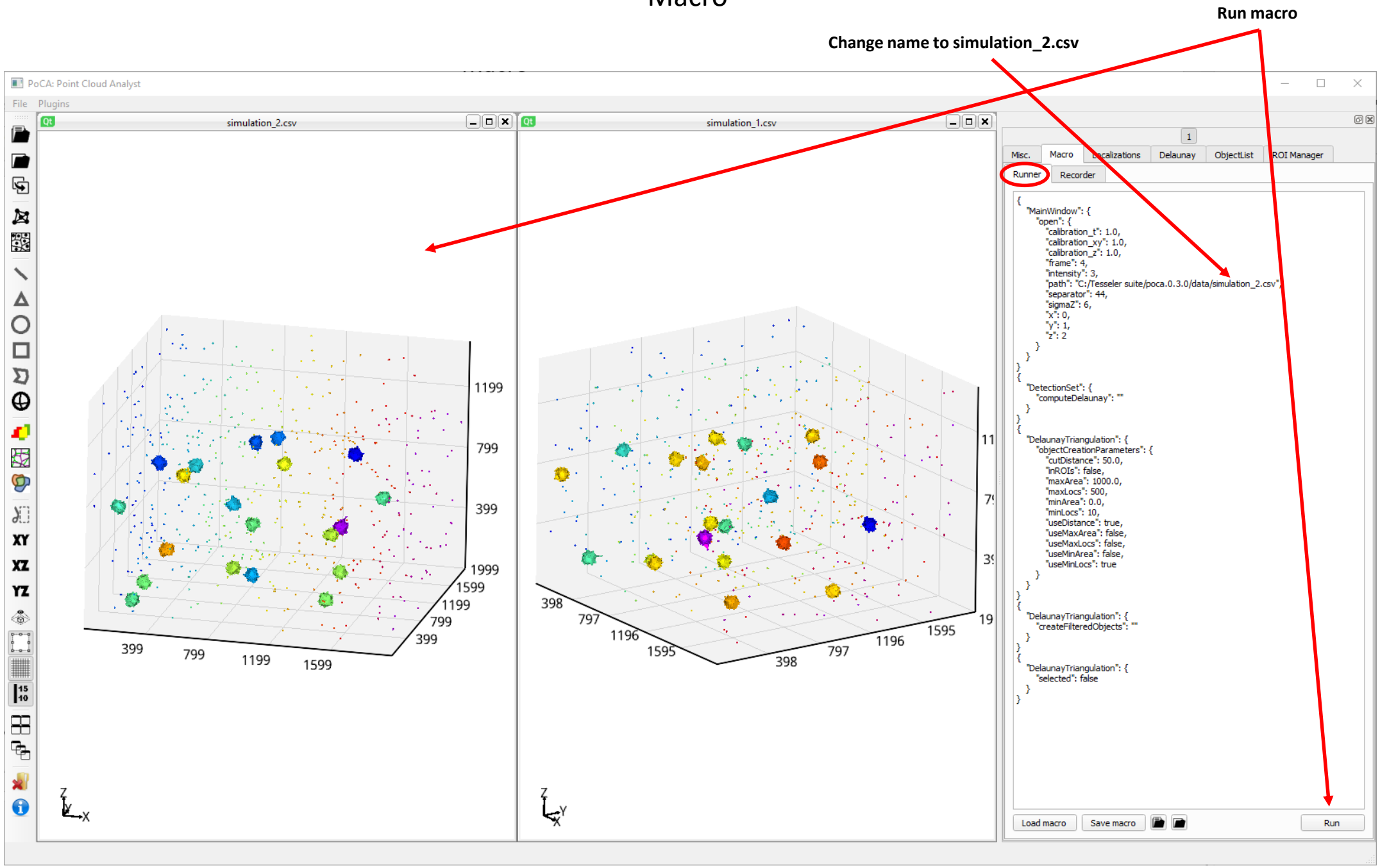
All these actions have been recorded in the Macro\\Recorder

Name of the dataset was simulation_1.csv

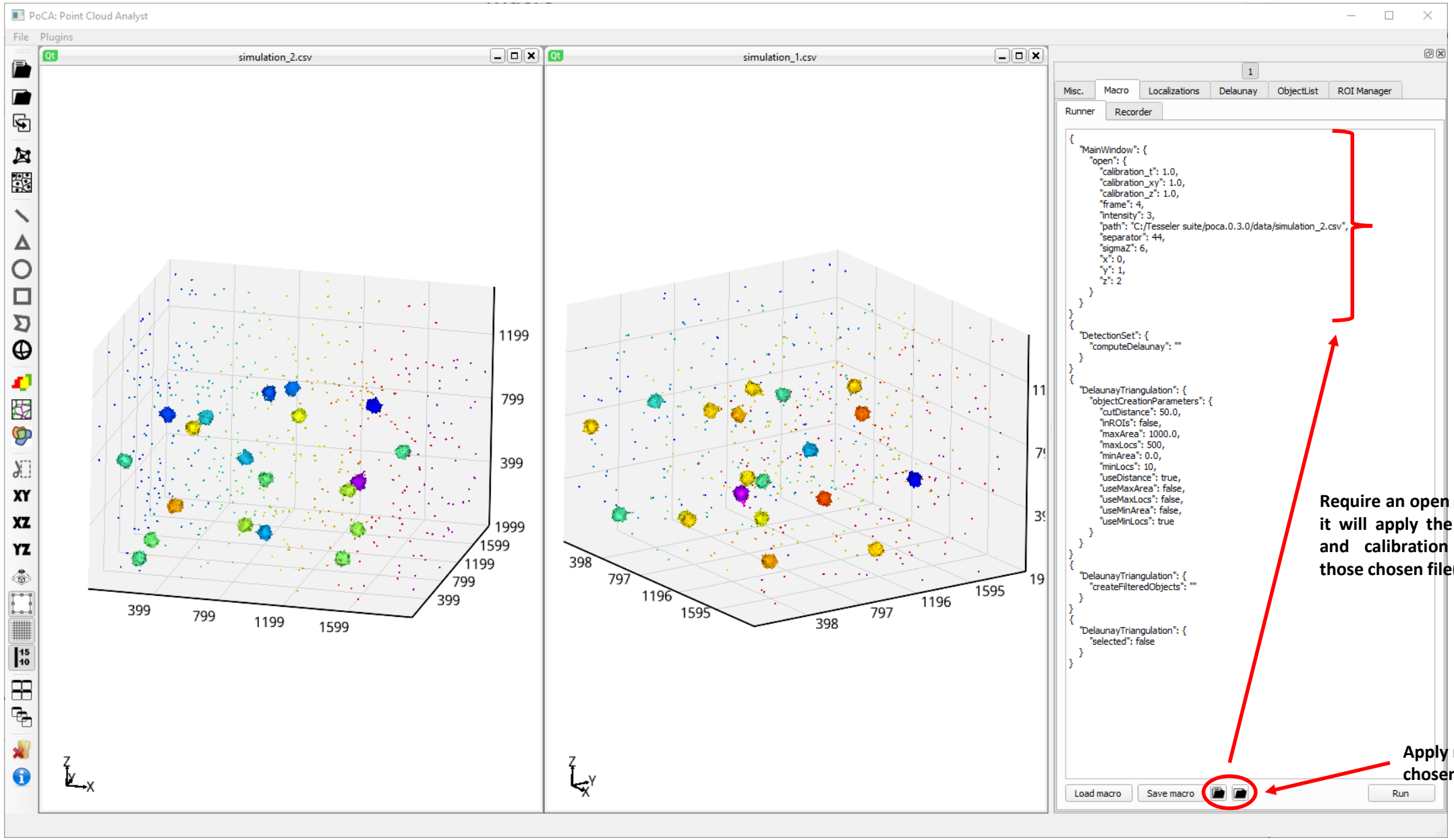
Transfer the recording to the runner



Macro


Run macro
Change name to simulation_2.csv
PoCA: Point Cloud Analyst
File
Plugins
simulation_2.csv
simulation_1.csv
Misc.
Macro
Localizations
Delaunay
ObjectList
ROI Manager
Runner
Recorder
{
"MainWindow": {
"open": {
"calibration_t": 1.0,
"calibration_xy": 1.0,
"calibration_z": 1.0,
"frame": 4,
"intensity": 3,
"path": "C:/Tesseler suite/poca.0.3.0/data/simulation_2.csv",
"separator": 44,
"sigmaZ": 6,
"x": 0,
"y": 1,
"z": 2
}
}
}
{
"DetectionSet": {
"computeDelaunay": ""
}
}
{
"DelaunayTriangulation": {
"objectCreationParameters": {
"cutDistance": 50.0,
"inROIs": false,
"maxArea": 1000.0,
"maxLocs": 500,
"minArea": 0.0,
"minLocs": 10,
"useDistance": true,
"useMaxArea": false,
"useMaxLocs": false,
"useMinArea": false,
"useMinLocs": true
}
}
}
{
"DelaunayTriangulation": {
"createFilteredObjects": ""
}
}
{
"DelaunayTriangulation": {
"selected": false
}
}
Load macro
Save macro
Run
XY
XZ
YZ
399
799
1199
1599
1999
398
797
1196
1595

Macro


PoCA: Point Cloud Analyst
File
Plugins
simulation_2.csv
simulation_1.csv
Misc.
Macro
Localizations
Delaunay
ObjectList
ROI Manager
Runner
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{
"MainWindow": {
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"calibration_t": 1.0,
"calibration_xy": 1.0,
"calibration_z": 1.0,
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"intensity": 3,
"path": "C:/Tesseler suite/poca.0.3.0/data/simulation_2.csv",
"separator": 44,
"sigmaZ": 6,
"x": 0,
"y": 1,
"z": 2
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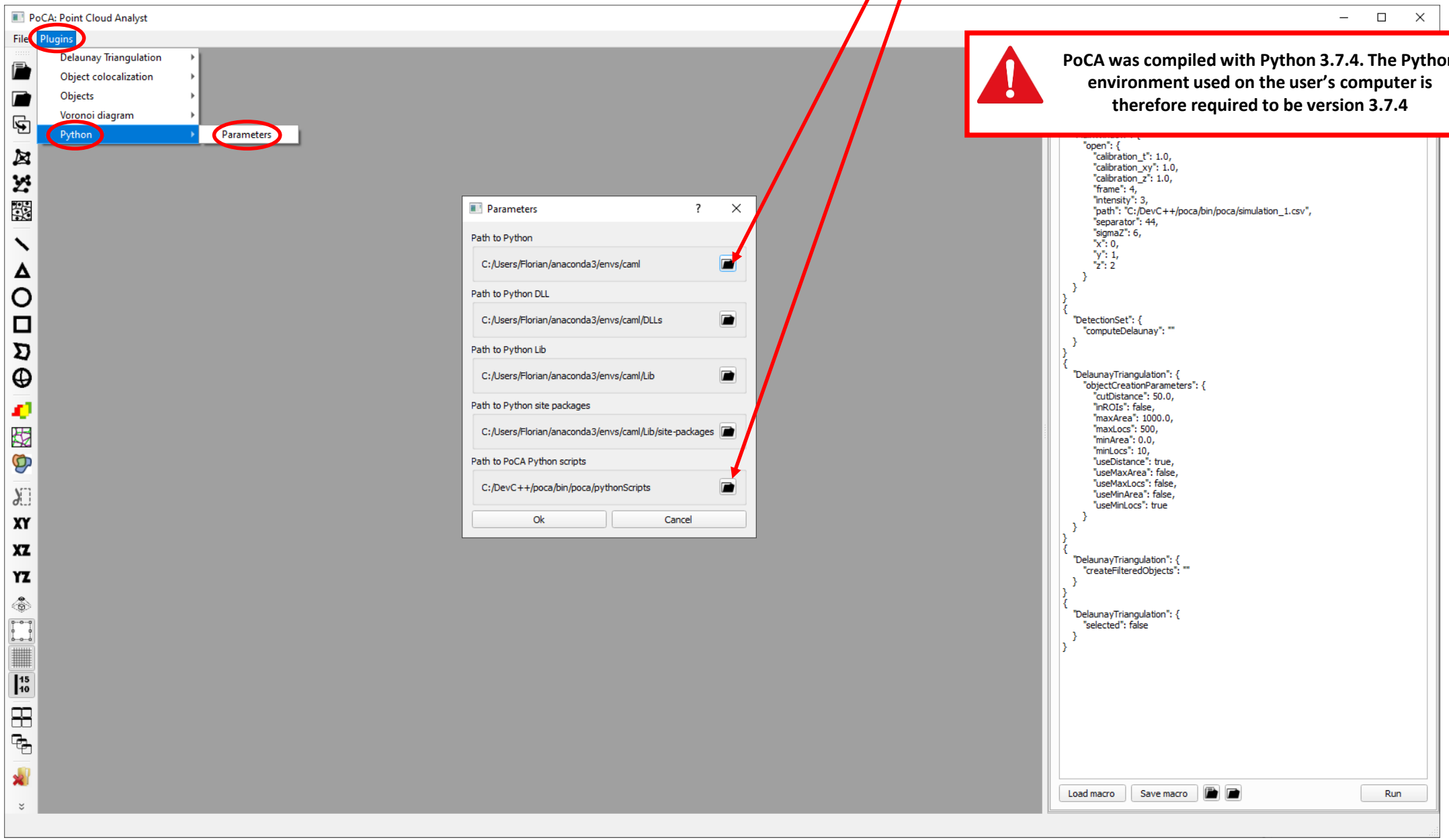
}
}
{
"DetectionSet": {
"computeDelaunay": ""
}
}
{
"DelaunayTriangulation": {
"objectCreationParameters": {
"cutDistance": 50.0,
"inROIs": false,
"maxArea": 1000.0,
"maxLocs": 500,
"minArea": 0.0,
"minLocs": 10,
"useDistance": true,
"useMaxArea": false,
"useMaxLocs": false,
"useMinArea": false,
"useMinLocs": true
}
}
}
{
"DelaunayTriangulation": {
"createFilteredObjects": ""
}
}
{
"DelaunayTriangulation": {
"selected": false
}
}
Load macro
Save macro
Run
Require an open command as it will apply the columns' id and calibration chosen on those chosen file(s)/folder
Apply macro to a chosen file or folder

Python

1st step is to define the different paths to Python and the scripts. Go to Plugins//Python//Parameters

Use folder icons to define the path, in the example we used an environment called CAML in Anaconda

Folder to PoCA Python scripts. It is mandatory that all those scripts are in this folder

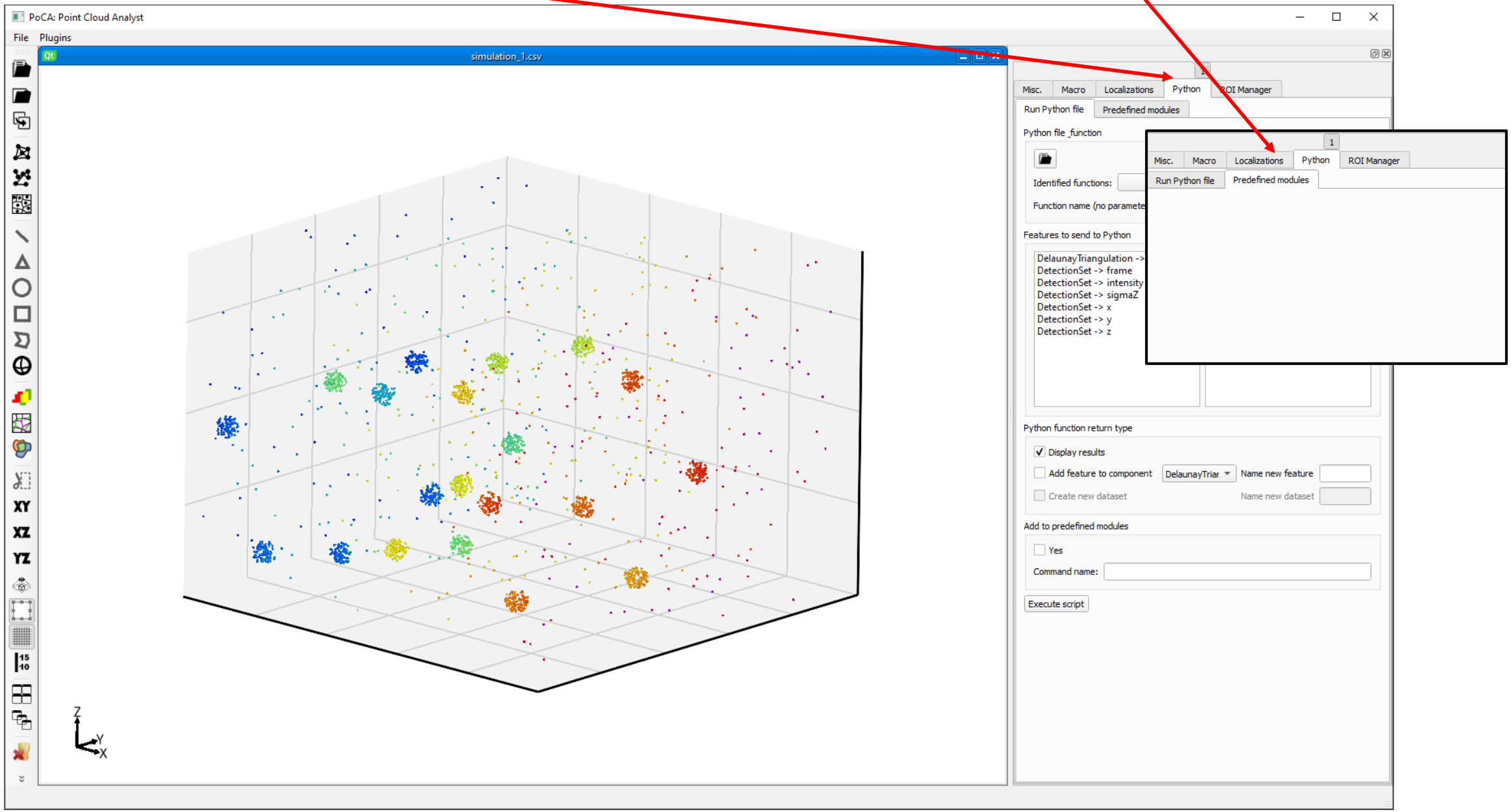


PoCA was compiled with Python 3.7.4. The Python environment used on the user's computer is therefore required to be version 3.7.4

Python

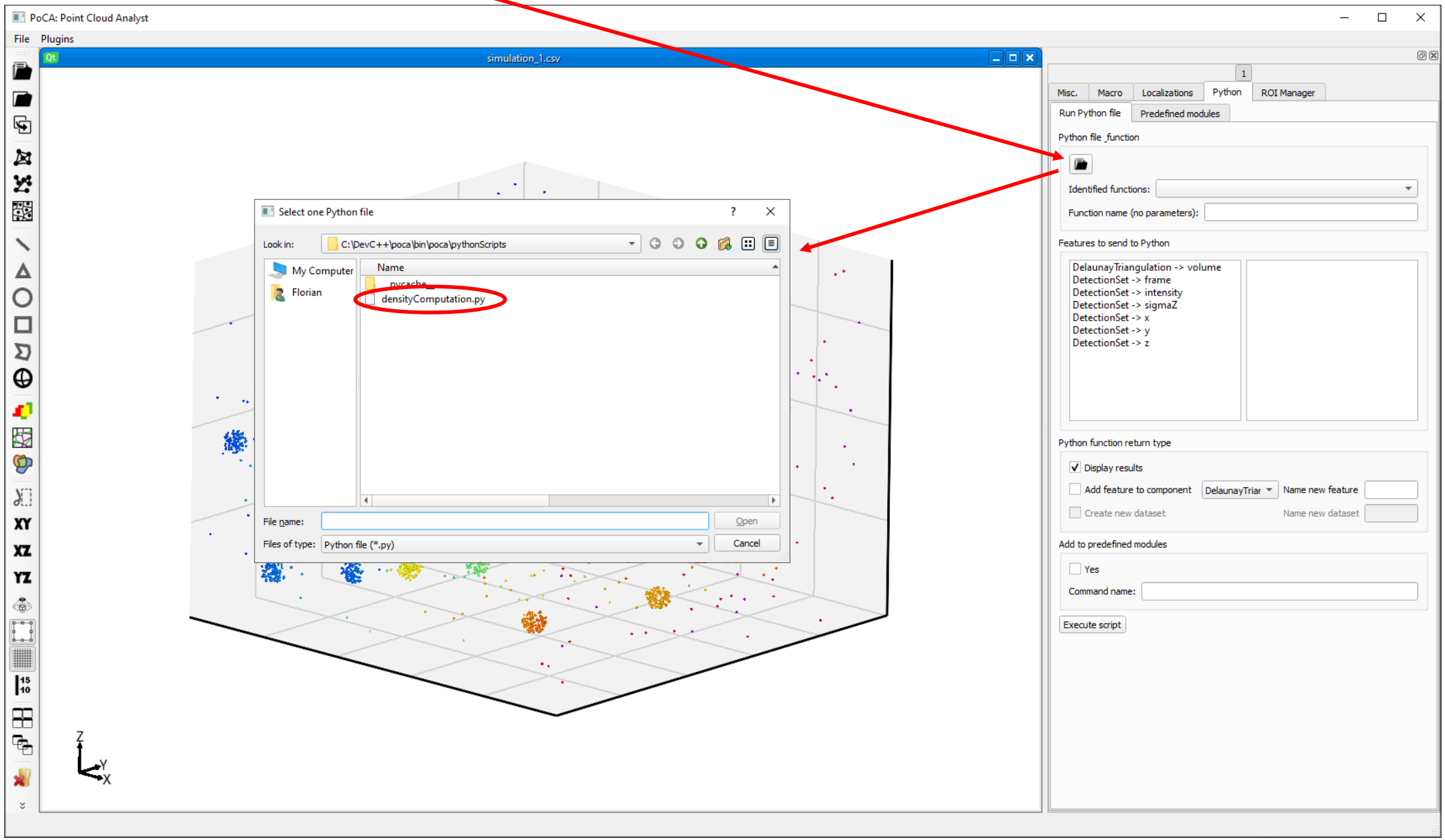
After loading a dataset, the Python tab is available

Predefined modules tab is currently empty



Python

Open the « densityComputation.py » Python file



Python

PoCA automatically identifies function name if the structure is « def funcName: »

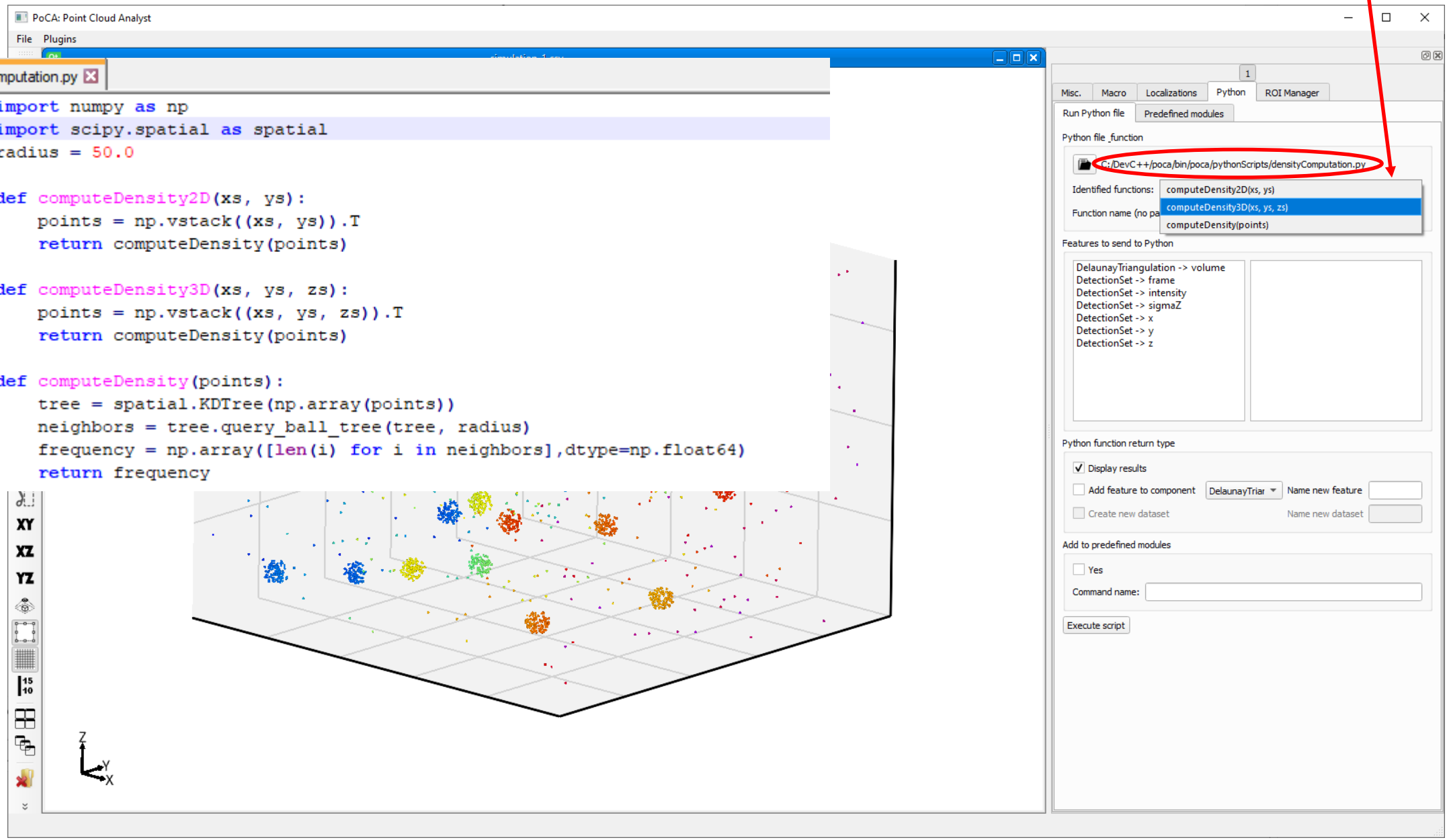
densityComputation.py

```python
import numpy as np
import scipy.spatial as spatial
radius = 50.0

def computeDensity2D(xs, ys):
    points = np.vstack((xs, ys)).T
    return computeDensity(points)

def computeDensity3D(xs, ys, zs):
    points = np.vstack((xs, ys, zs)).T
    return computeDensity(points)

def computeDensity(points):
    tree = spatial.KDTree(np.array(points))
    neighbors = tree.query_ball_tree(tree, radius)
    frequency = np.array([len(i) for i in neighbors],dtype=np.float64)
    return frequency
```



Python

Currently, PoCA can only transfer 1 dimension arrays to Python
So only computeDensity2D & computeDensity3D can be used
computeDensity is an internal function that have a multidimensional array as input

Choosing a function automatically fills the function name
If the function was not in the list, it can be filled by the user in the text edit

densityComputation.py

```python
import numpy as np
import scipy.spatial as spatial
radius = 50.0

def computeDensity2D(xs, ys):
    points = np.vstack((xs, ys)).T
    return computeDensity(points)

def computeDensity3D(xs, ys, zs):
    points = np.vstack((xs, ys, zs)).T
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    return frequency
```

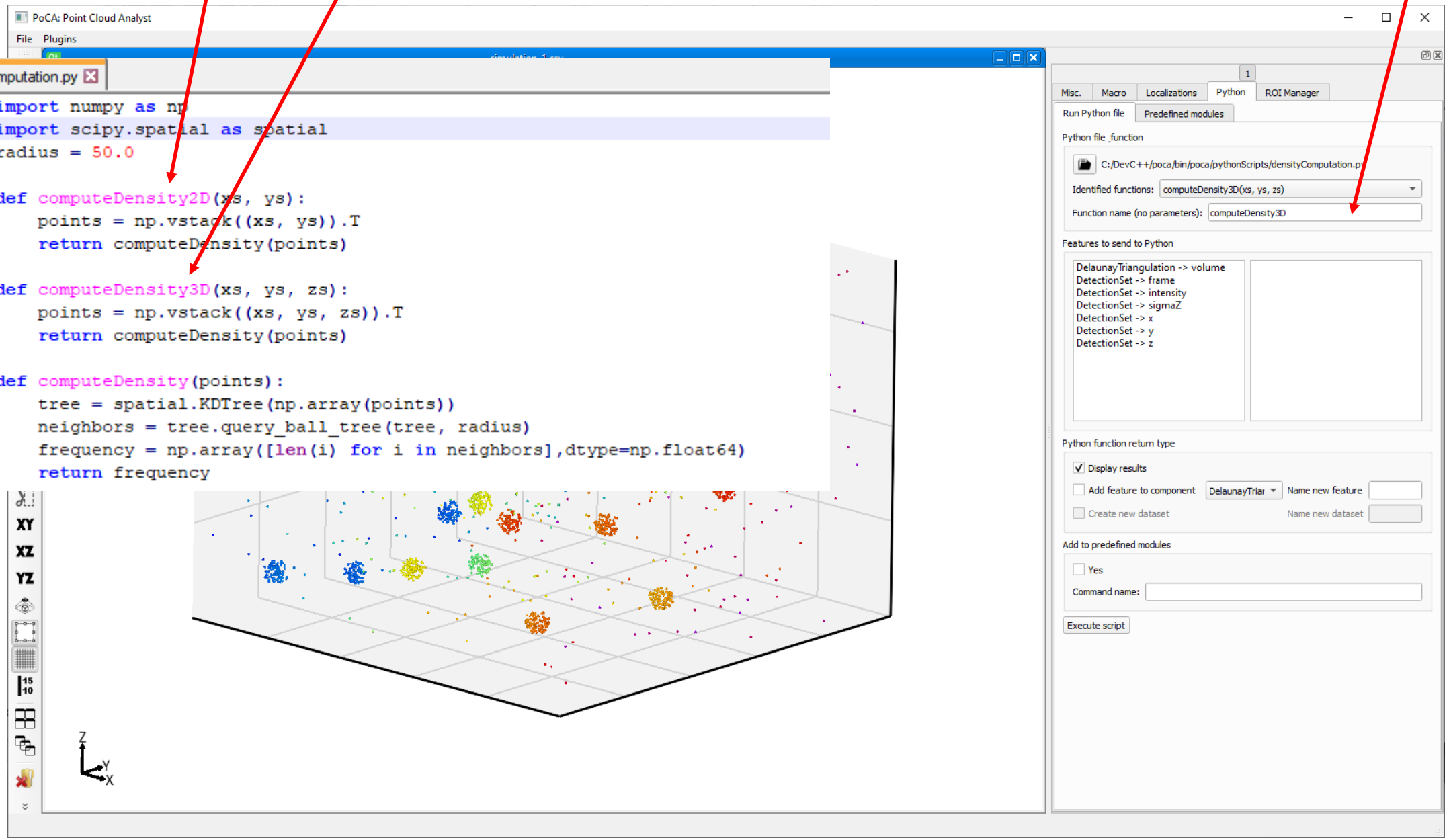

Python

computeDensity2D & computeDensity3D both return 1 dimensional array (the computed density)

Select « add feature to component »

Select the correct component

Name the added feature

densityComputation.py

```python
import numpy as np
import scipy.spatial as spatial
radius = 50.0

def computeDensity2D(xs, ys):
    points = np.vstack((xs, ys)).T
    return computeDensity(points)

def computeDensity3D(xs, ys, zs):
    points = np.vstack((xs, ys, zs)).T
    return computeDensity(points)

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    neighbors = tree.query_ball_tree(tree, radius)
    frequency = np.array([len(i) for i in neighbors],dtype=np.float64)
    return frequency
```
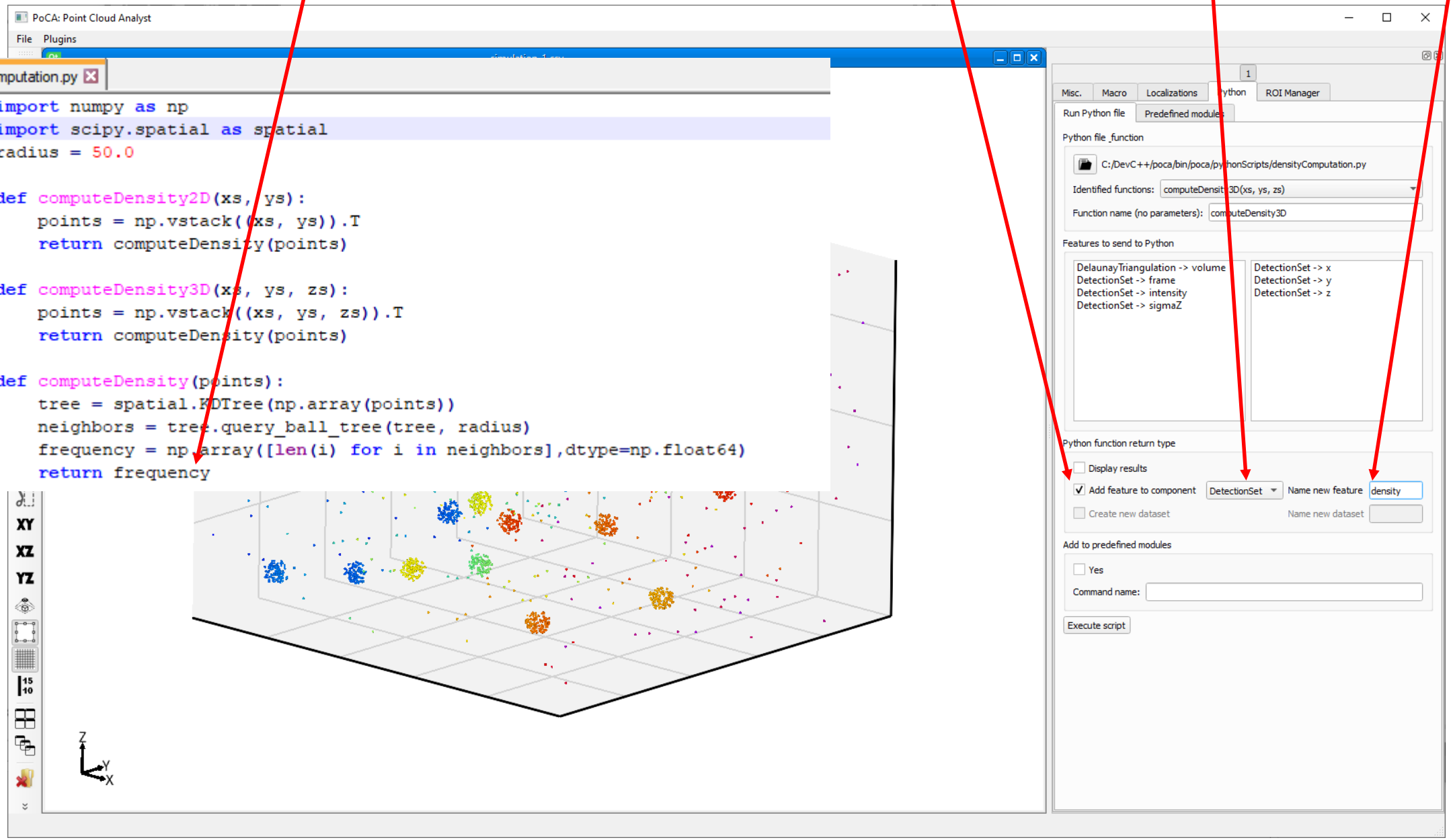



Python

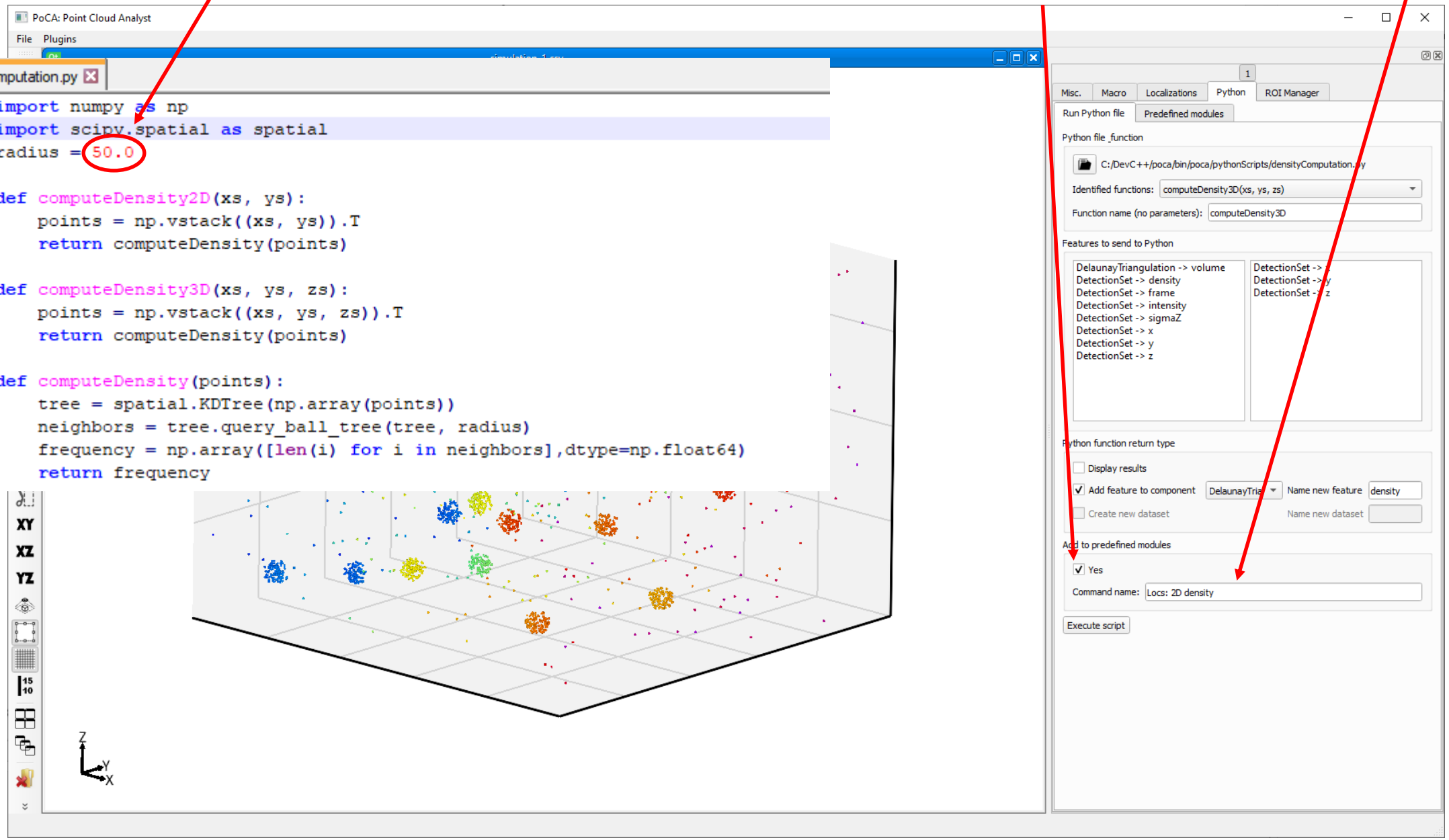
ComputeDensity computes, for each loc, the number of points in its vicinity defined by radius
Radius is hard-coded in the script

Any Python script can be added as predefined module, check « Yes »

Give a name to the Python command

densityComputation.py

```python
import numpy as np
import scipy.spatial as spatial
radius = 50.0

def computeDensity2D(xs, ys):
    points = np.vstack((xs, ys)).T
    return computeDensity(points)

def computeDensity3D(xs, ys, zs):
    points = np.vstack((xs, ys, zs)).T
    return computeDensity(points)

def computeDensity(points):
    tree = spatial.KDTree(np.array(points))
    neighbors = tree.query_ball_tree(tree, radius)
    frequency = np.array([len(i) for i in neighbors],dtype=np.float64)
    return frequency
```



Python

Computed density have been added to the localizations

The script has been added as a predefined module. It should be loaded on the next PoCA lauch (if PoCA was properly closed ; with the cross)