**Clus-DoC Output files**

**Input files** (all in one folder)

* .txt files generated from Zen containing x,y coordinates of all your points, one file per cell (e.g. 1.txt, 2.txt, 3.txt etc.)
* Optional: coordinates txt file containing centre coordinates of ROIs for analysis (4 tab-delimited columns required: 1st column: ROI name, 2nd column: cell number, 3rd/4th column: x and y coordinates respectively).
* Other option is to load the cells in the GUI and select ROIs directly in the GUI.

**DoC histograms** (folder)

* contains histograms of DoC scores of all molecules for each ROI and for all pooled data
* Distribution of DoC scores for Channel 1 (top; green) and for Channel 2 (bottom; red)

**DoC Statistics and Plots** (folder)

* contains 3 folders:
* 2 with raw data/raw data without outliers (good representative images of what the raw data looks like in each ROI). Ch1 is in green, Ch2 is in red.
* 1 folder with density maps and DoC maps for Ch1 and Ch2 for each ROI.
* Density maps: relative normalised density maps (normalised to 1; relative to total density)
* DoC maps: all molecules are colour-coded according to their DoC scores

**DBSCAN Results** (folder)

* contains DBSCAN cluster analysis for Ch1 and Ch2 separately
* in Ch1 and Ch2 folders: cluster maps of ROIs for Ch1 and Ch2 separately (non-clustered molecules are in gray; molecules in clusters are in green/red for Ch1/Ch2 respectively; cluster contours are drawn in black).
* DBSCAN Results excel file: cluster parameters for Ch1 and Ch2 in each ROI (different sheets for Ch1 and Ch2)

**Clus-DoC Results** (main results folder)

* DoC Results excel file: contains percentage of Ch1 molecules that are colocalised with Ch2 / percentage of Ch2 molecules that are colocalised with Ch1 – for each ROI (based on a DoC threshold of 0.4 but this can be changed by the user)
* Clus-DoC Ch1/Clus-DoC Ch2 excel files: contain parameter information (relative density, area, circularity, average number of molecules per cluster) for colocalised versus non-colocalised clusters (a colocalised cluster is defined as having more than 10 molecules with DoC≥0.4) – average for each ROI
* Gives information about what clustering parameters in one channel make it more likely to be colocalised with the other channel.

**Export files** (saved in main input data folder)

* At the end of your analysis, click ‘Export Results Table’ button in the GUI if you want values on a per point or per cluster basis for further analysis.
* For each cell (x.txt file), you get a file called ‘x\_ExportByPoint’ where data for every point from the analysed ROIs is saved (e.g. DoC score for all points)
* You also get a ‘ClusterExport’ file containing information on a per cluster basis.