Polygon-based colocalization analysis for multicolor single-molecule localization microscopy data

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Over the last decade, single-molecule localization microscopy (SMLM) has revolutionized cell biology, making it possible to monitor molecular organization and dynamics with spatial resolution of a few nanometers. When used with multiple colors, it enables the investigation of potential interactions between subcellular components at the nanoscale. However, while colocalization analysis has been thoroughly used on pixel-based images, most of these techniques are not adapted to the pointillistic nature of SMLM data. More generally, quantification of SMLM data has proven to be particularly complex due to several experimental parameters influencing localization densities (e.g. fluorophore photophysics, labeling density, acquisition time, localization errors, etc.).

We recently developed a segmentation and quantification method for 2D SMLM data, distributed as an open-source software called SR-Tesseler [1]. In this framework, localization coordinates are directly used to compute a Voronoï tessellation, partitioning the image space in polygons of various sizes centered on each molecule. Here, we present Coloc-Tesseler [2], a software in which we extend the intrinsic multiscale capabilities of the Voronoï tessellation with a new metric in order to perform normalized colocalization analysis of multicolor SMLM data. Using simulation and experimental data, in 2D and 3D, we have demonstrated that such a method can achieve robust analysis of multicolor SMLM data with different relative molecular densities.

[1] F. Levet et al, *SR-Tesseler: a method to segment and quantify localization-based superresolution microscopy data*, Nature Methods, 12 (11); 1065-1071 (2015).
[2] F. Levet et al. *A tessellation-based colocalization analysis approach for single-molecule localization microscopy.* Nature Communications (10), Article number: 2379 (2019).