

# SEGMENTATION AND CLUSTER ANALYSIS OF PROTEIN COMPLEXES BY VORONOI TESSELLATION IN 3D LOCALIZATION MICROSCOPY

Leonid Andronov, Jonathan Michalon, Khalid Ouararhni, Igor Orlov, Yves Lutz, Ali Hamiche, Jean-Luc Vonesch, Bruno Klaholz

Centre for Integrative Biology (CBI)  
Institute of Genetics, Molecular and Cellular Biology (IGBMC)  
Centre National de la Recherche Scientifique (CNRS)  
University of Strasbourg  
1 rue Laurent Fries, BP 10142, 67404 Illkirch CEDEX, France  
E-mail: andronov@igbmc.fr

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As opposed to classical light microscopy, the data of single-molecule localization microscopy is essentially discontinuous because it comprises a set of points with molecular coordinates of the localization events and hence it does not allow a direct segmentation. Considering the plethora of fluorescent signals in dense environments such as chromatin, the interpretation of localization data can become rather challenging with regards to image segmentation, clustering and colocalization analysis.

We have recently developed ClusterViSu, a robust pipeline for processing of 2D localization data based on Voronoi tessellations [1, 2]. ClusterViSu is suitable for the convenient visualization and quantification of the localization and distribution of fluorescently labeled complexes, which allows segmentation, cluster analysis and estimation of the amount of co-localization. Voronoi diagrams allow performing a statistical analysis of the clusters, their occurrence and inter-cluster distance distribution, and work well including for the analysis of weak signals.

We now demonstrate an extension of this method to address 3D single-molecule localization microscopy data. We show that 3D Voronoi diagrams can be useful for precise local density estimation, noise reduction, rendering and cluster analysis of 3D data. One of the advantages of the Voronoi tessellation method is that it does not require any a priori knowledge for the clustering. Because the clustering uses an internal reference generated with Monte-Carlo simulations of randomized data to automatically determine the threshold value for forming clusters between neighboring Voronoi zones, it is fully automated for a given region of interest.

The general applicability of the method is illustrated on microtubules, histones H2B and CENP-A chromatin data.

[1] L. Andronov; I. Orlov; Y. Lutz; J.-L. Vonesch, and B.P. Klaholz, "ClusterViSu, a method for clustering of protein complexes by Voronoi tessellation in super-resolution microscopy," *Sci. Rep.* **6**, 24084 (2016).

[2] L. Andronov; Y. Lutz; J.-L. Vonesch, and B.P. Klaholz, "SharpViSu: integrated analysis and segmentation of super-resolution microscopy data," *Bioinformatics* **32**(14), 2239-2241 (2016).