

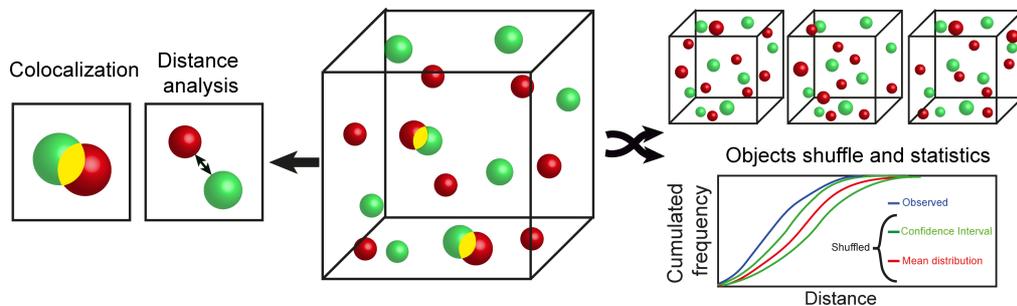
DiAna, an ImageJ tool for object-based 3D co-localization and distance analysis

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Many biological studies depend on the analysis of the distribution and spatial relationship between biomarkers in a cell or tissue. Various methods, from immunodetection to transgene-driven expression of fluorescent proteins, allow to observe positive cells in whole specimen or tissue sections at the histological level, or to detect protein sub-cellular localization at the cellular level. The 3D-organization and relationship of these biomarkers can be investigated using fluorescence microscopy techniques that allow optical sectioning, such as confocal microscopy or multiphoton microscopy, and should be combined with appropriate image analysis methods. Biologists often seek at associating the spatial overlap of pairs of biomolecules in a cell or in a sub-cellular compartment by the means of co-localization analysis, which is an indispensable tool before inferring biological interaction and drawing functional conclusions. Co-localization analysis has become an extensive field of research, it has been extensively reviewed and a plethora of open-source tools exist for the cell biologist [1, 2]. Besides, the cell biologist might be interested in further associations of neighboring structures, such as the proximity of two markers.



We provide a tool that includes a thorough object based co-localisation analysis, which is combined with the possibility to analyse further spatial relationships between sub-cellular structures, such as proximity.

In our approach, we guide the user to obtain segmented images to quantify his data. We created a ImageJ-based tool, DiAna (**D**istance **A**nalysis) that provides different possibilities for spatial analysis, object colocalisation, distances between the two populations of object and statistics on each object [3]. With DiAna, we furthermore introduce an original method, which allows for estimating the statistical significance of object co-localization. In addition to result tables, we introduce an ROI selector to navigate through images and the possibility to save them. DiAna thus offers a complete and intuitive 3D image analysis tool for biologists.

[1] Bolte S and Cordelieres FF (2006) J Microsc 224(Pt 3):213-32, [2] Cordelières FP and Bolte S (2014) Methods Cell Biol. 2014; 123:395-408, [3] Gilles JF et al., (2016) Methods, pii: S1046-2023(16)30464-9