

# SPATIAL STATISTICS IN BIOIMAGING WITH SPATIAL OBJECT DISTRIBUTION ANALYSIS (SODA)

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To elucidate the cellular processes and host-pathogen interactions, it is important to localize and quantify the spatial distributions of different objects (molecules, organelles...) at (sub)cellular level. This is commonly performed with microscope visualization and is generally achieved through the study of the spatial colocalization between different populations of molecules labeled with different fluorophores (in general red and green fluorophores). Most colocalization methods are based on pixel overlap between the previously denoised signal that is emitted from two (or more) different fluorescent labels, and use a global image correlation such as Pearson's or Manders' coefficients [1]. These data, however, cannot be linked to physical parameters such as the real percentage of colocalizing molecules or the average colocalization distance. In addition, randomly distributed molecules can partially overlap, and it is hard to measure the statistical significance of the computed correlation indices. Moreover, the traditional measure of signal overlap or correlation, i.e. the “yellow” amount of an image, is made obsolete by advances in microscopy and increased spatial resolution. Moreover, overlap analysis depends on microscope point-spread-function (PSF) and cannot be extended to other bioimaging modalities, such as electron microscopy or colored histopathological samples. Therefore, we have developed a method and software named Statistical Object Distance Analysis (SODA) to characterize the relative spatial positioning of several distributions of objects in a quantitative and automatic manner [2]. Our method is based on the automatic detection of molecule fluorescent spots, followed by their representation as Point Processes and the statistical analysis of their spatial distribution. We use the mathematical framework of point processes, the method does not depend on the PSF characteristics and can be used with any imaging modality. In addition, by analyzing the morphology (size, intensity and shape) and the distance separating coupled objects, it provides, at a population level, a detailed and exhaustive map of objects' relations. For example, using SODA with three-color structured-illumination microscopy (SIM) images of hippocampal neurons, we statistically characterized the spatial organization of thousands of synapses. We will illustrate the method further through examples in TIRF and 3D-STORM microscopy.

[1] Bolte, S. and Cordelieres, F. P. (2006) A guided tour into subcellular colocalization analysis in light microscopy. *J. Microscopy*, 224, pp. 213–232

[2] Lagache, T., Grassart, A., Dallongeville, S., Faklaris, O., Sauvonnnet, N., Dufour, A., Danglot, L., Olivo-Marin, J.-C. (2018) Mapping molecular assemblies with fluorescence microscopy and object-based spatial statistics, *Nature Communications*, 9,1, pp. 698-711. doi: 10.1038/s41467-018-03053-x.