A Point Process Analysis Framework for Quantitatively Describing Spatial Patterns in Fluorescence Microscopy Data

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Abstract
The current standard practice for assessing the relative spatial association between fluorescently colabeled biological targets is the pixel intensity paradigm, where colocalization of different fluorescence signals is characterized by comparing the brightness of individual pixels across their spectral channels using Manders’ co-occurrence or Pearson’s correlation coefficients. Though popular and easy to implement, this approach is critically limited due to its high sensitivity to labeling and imaging procedures, low descriptive power, dependence on image resolution, and inability to assess spatial relationships beyond direct overlap. Here, we propose an improved framework for quantitatively assessing relative spatial patterns from microscopy data that overcomes these limitations by leveraging the point process analysis paradigm.

Spatial point process data are those that describe the positions of events that are the spatial realization of some underlying process. One flavor of point process analysis aims to describe the spatial properties of such processes using distribution functions derived from the nearest neighbor distances (NND) between heterologous events. Specifically, the spatial association between two species of events is characterized by comparing their cumulative distribution function (CDF) of observed NNDs to a null hypothesis distribution, often the CDF of NNDs generated by simulated events whose positions are random samples from the uniform distribution. Significant deviation of the observed CDF from this random CDF indicates a departure of the observed process from spatial randomness, thus exhibiting some degree of either aggregation or dispersion between event species.

We have adapted this paradigm for image data to describe the spatial association between pairs of labeled biological targets. For example, we can evaluate the difference in association to a cellular landmark, such as the nuclei of and isolated cardiomyocytes, between two biomolecule subjects, such as mRNA encoding for the messenger protein calmodulin (Calm3) and voltage-gated sodium ion channel Na1.5 (Scn5a) (Fig. 1A). First, each labeled target’s events are identified by segmenting their true signal from their respective spectral channels, the final product of which can be visualized as a binary image mask (Fig. 1B). Then, NNDs between subject and landmark events are calculated by intersecting the subject’s mask with the Euclidean distance transformation of the landmark’s mask (Fig. 1C). The subject’s spatial association with the landmark is then assessed by comparing its CDF of observed NNDs to the landmark’s random NND CDF generated from the NNDs between landmark events and a random sample of intracellular pixels (random events) (Fig. 1D-F). The signed test statistic from a 2-sided Kolmogorov-Smirnov test comparing observed and random CDFs (Fig. 1G) indicates the type of association between subject and landmark, where a positive value indicates subject aggregation with the landmark (subject-landmark NNDs are greater than those expected if the subject were truly randomly distributed), a negative value indicates subject dispersion from the landmark (subject-landmark NNDs are greater than those expected if the subject were truly randomly distributed), and a value at or near zero indicates no spatial association between subject and landmark (subject-landmark NNDs match those expected if the subject were truly randomly distributed).

Because this analysis relies on inter-signal distances rather than their direct overlap, as in colocalization paradigms, it is free to assess association at all spatial scales contained within the image. Additionally, its results are uninfluenced by imaging resolution as long as the resolution is greater than the spatial scale of the process being studied. Finally, different null hypothesis distributions can be substituted to further probe the spatial relationship between labeled biological targets. Our framework offers significant improvements over standard spatial analyses due to its greater descriptive power, robustness, and modularity.

Example application of point process analysis on (A) a fluorescence microscopy image of an isolated cardiomyocyte labeled for Calm3 mRNA (red), Scn5a mRNA (green), and nuclei (white) – gray indicates the cell body and yellow where the two mRNA species overlap. (B) These signals are segmented to identify their events and (C) NND are calculated using the nuclei mask’s Euclidean distance transformation. (D-E) Distribution functions of these distances are calculated and (F) compared to a simulated random distribution to determine (G) the mRNAs’ spatial association to the cell’s nuclei. This cell’s observed mRNA distributions are significantly different from one another and from the nuclei’s random distribution, indicating that Calm3 mRNA is significantly dispersed from the nuclei and Scn5a mRNA is significantly aggregated with the nuclei.

References