CLUSTER ANALYSIS METHODS AND CORRELATIVE MICROSCOPY TOOLS FOR LOCALIZATION MICROSCOPY

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Single Molecule Localization Microscopy (SMLM) can provide high specificity images of proteins in their natural conditions, with single molecule resolution. However, it has several sources of errors, limiting its utility for quantitative measurements. For example, PhotoActivation Localization Microscopy (PALM) can typically detect only about 40-60% of the fluorophores in the sample, and that it has a measurement error (FWHM) in the order of 25-50nm[1].

I will present a cluster analysis framework that accounts for the errors introduced by the limited detection efficiency and localization precision that are implicit in PALM [2]. The work demonstrates that the commonly used analysis tools Ripley L(r)-r function and the Pair Correlation Function are invariant to spatial subsampling and hence detection efficiency. We present analytical solutions for the mean and variance of these functions of the subsampled points, and also a method to estimate the true point locations in clusters corresponding to the localizations with uncertainty. Figure 1 shows an example result based on a simulated point pattern (density: 800um^-2), where the method presented could estimate the true L(r)-r functions with high accuracy and precision from noisy observations (detection efficiency of 50% and mean localization precision of 18nm).

I will also briefly present our results on another collaborative project (LBNI lab, EPFL) on an SMLM-AFM correlated microscopic tool, so that the SMLM data can be validated with AFM data, and its various errors characterized [3]. STORM-AFM correlated images of in vitro actin filaments and bacteria will be presented.