Chemical & Physical Sciences
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Colloquium Seminar Series
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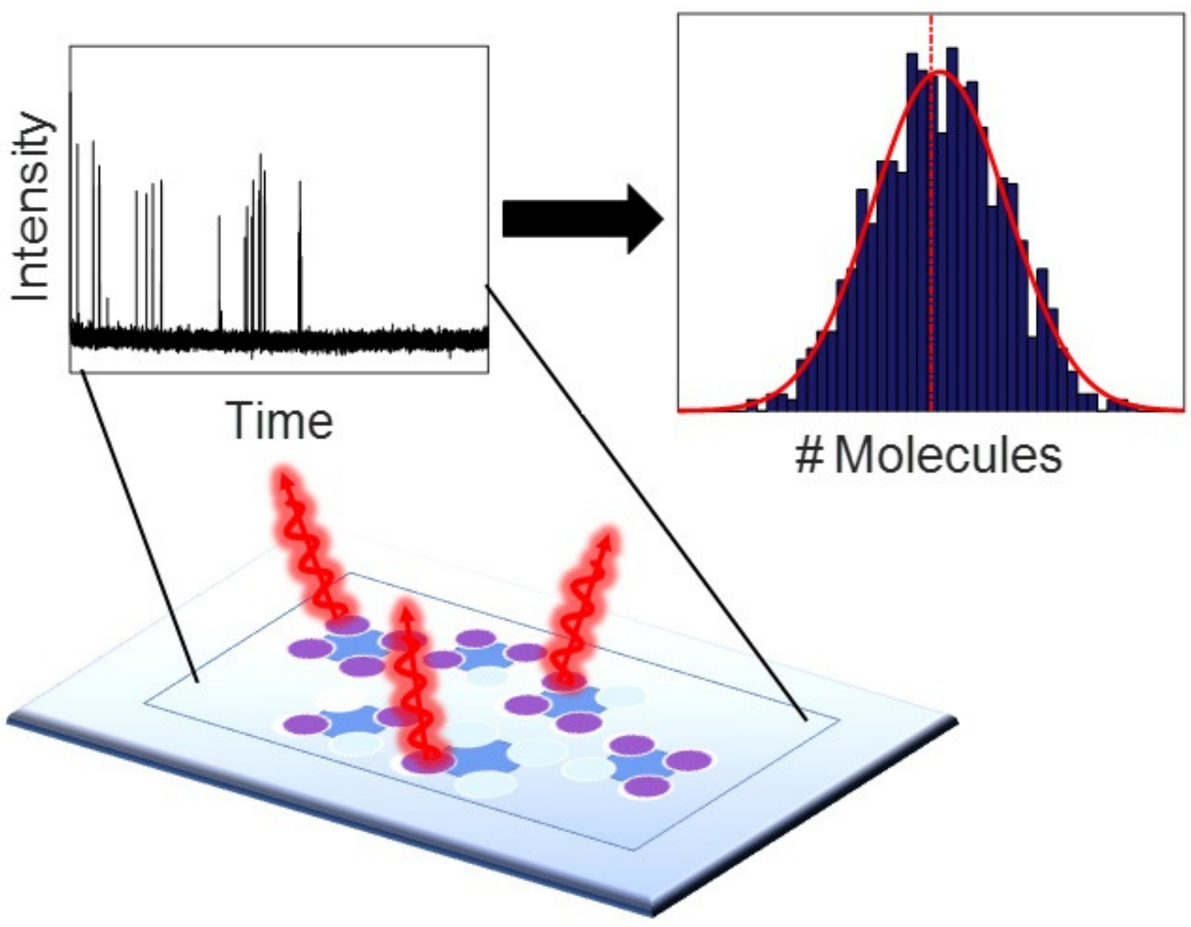
On the molecular counting and clustering problems in single-molecule quantitative nanoscopy



The advent of single-molecule localization microscopy (SMLM) revealed the nanoscopic domain of cellular and molecular biology beyond the diffraction-limit of light. Since its inception, much effort has been focused on the continued improvement of SMLM as a qualitative tool for the study of biological systems that were previously out of reach by fluorescence microscopy. However, a complete understanding of these systems also requires the development of techniques that can extract quantitative information to complement the imaging capabilities of SMLM.

This talk will present the development of a molecular counting technique based on a Bayesian analysis of the blinking statistics of fluorophores in SMLM. This method considers labeling distributions which result from tagging techniques used to attach fluorophores to biomolecules. The accuracy of the technique in determining molecule copy numbers and protein stoichiometry is then experimentally demonstrated using two constructs of DNA origami grids to mimic biomolecules of interest. The dynamic range (allowed fluorophore densities) of counting methods based on SMLM is also briefly studied and determined to be on the order of hundreds of fluorophores per diffraction-limited area.

Additionally, the development of a density-based three-dimensional cluster analysis algorithm (FOCAL3D) for SMLM is outlined. FOCAL3D scans for optimal values of its parameters via a semi-supervised, data-driven pipeline, and is able to perform fast and accurate identification of clusters better than many existing clustering algorithms, most notably the popular DBSCAN algorithm.

These contributions provide new tools for the extraction of quantitative information from biological systems imaged by SMLM.