photobleaching, thus leading to a set of localizations scattered around the true position. Whereas this property does not affect the reconstruction of images of extended objects like intracellular filaments or compartments, it severely influences the interpretation and quantification of objects for which the exact stoichiometry can be important like for membrane protein aggregates. To resolve this issue, we have implemented an algorithm that uses the spatial and temporal information of fluorophore localizations from STORM/PALM experiments to obtain a quantitative picture of the underlying molecule distribution. Our algorithm reliably operates on artificial data as well as on experimental data from biological constructs with a well-defined number of attached fluorophores.

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Regulating Bacterial Cytokinesis: A Super-Resolution Study of ZapA and ZapB

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E. coli cell division is achieved by the concerted effort of at least 13 essential proteins that assemble at midcell in a cell-cycle dependent manner to form a macromolecular structure (divisome) capable of generating septal force. Central to divisome assembly is the initial formation of the Z-ring, a dynamic, cytokinetic suprastructure of the FtsZ protein, the prokaryotic tubulin homolog widely conserved in bacteria. Being the first division protein to localize precisely to midcell, spatial and temporal regulation of FtsZ polymerization is of critical importance to efficient proliferation.

From conventional fluorescence microscopy, the Z-ring has long been regarded as a closed-ring that circumscribes the cell. However, in vitro studies illustrated FtsZ's tendency to self-assemble into short, single-stranded protofilaments that further coalesce into multi-stranded rings and helices under molecular crowding conditions. Recently, our group has shown that the relevant in vivo structure of FtsZ is characterized by an irregular, discontinuous arrangement of overlapping protofilaments, observed in a closed ring as well as a compressed helical conformation. We believe that an equilibrium exists between the helix and ring conformations and therefore factors promoting the transition from helix to ring may serve as critical regulatory elements or pathways.

ZapA and ZapB are both non-essential, cytoplasmic proteins that associate directly with FtsZ early during division. Although their null mutants have little observable cytokinetic defect, both have a pronounced prevalence of FtsZ helical conformations, indicating their presence favors ring formation. In this study, we employ super-resolution imaging to characterize the relevant in vivo arrangements of ZapA and ZapB, as well as their relative localization with respect to FtsZ using two-color imaging methods. Lastly, by characterizing the helical conformation the Z-ring in the Zap-null mutants, we gain insight to their potential regulatory mechanism.

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Corral Trapping of Single Molecules in Solution: Theory and Applications

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One of the great outstanding challenges for the fabrication of nanosensors and nanodevices that will drive future technologies and enable the planned assembly of molecular-scale devices is the controlled manipulation of nanoscale objects, and particularly of single molecules. Any such manipulation is preceded by an efficient, reliable method for confining (trapping) an object on demand, which has remained a formidable task in the case of single molecules.

We have successfully trapped single molecule ions and other charged particles in aqueous electrolytes using a purely electrostatic setup, demonstrating stable and reversible confinement of single and multiple particles to nanoscale dimensions over extended periods of time.

Here, we will present some of the experimental results and discuss potential mechanisms for corral trapping. Electrokinetic phenomena such as electrohydrodynamic flow, electroosmotic flow, or dielectrophoresis are known to occur near a charged metal electrode; however, we attribute corral trapping to direct charge-field interactions, i.e. and electrostatic or electrophoretic mechanism, which seems consistent with all experimental observations. Theoretical modeling of the entire setup using the finite element method will be discussed, and the limitations for applications in SNP detection and water treatment technologies will be explored.

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A Rejection Algorithm Essential for Quantitative Analysis in Single Molecule Super Resolution Microscopy

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Single molecule localization based super-resolution (SM-SR) imaging techniques require robust identification and accurate localization of single molecules to yield reliable emitter position estimates for further quantitative analysis. One of the key steps of SM-SR analysis is to clearly identify isolated, single emitters such that obtained localizations relate to actual single molecule locations, and are not influenced by signal from nearby or overlapping fluorophores. We refer to the process of identifying and discarding sub-regions that contain overlapping fluorophores as the rejection algorithm.

We show that even at an optimal active emitter density, more than 50% of the emitters cannot be isolated such to give an unbiased estimate of position. Furthermore, it is possible that even for the case of isolated single emitters, the fitting routine may not converge and thus provides incorrect localizations. A robust, statistically rigorous rejection algorithm that compares the fitted model to the data is necessary to avoid incorrect or biased localizations and is essential for the success of quantitative analysis.

Here, we show that the Log-likelihood Ratio (LLR) is a preferred metric for rejection algorithms and that p-values can be calculated and used for statistical significance tests when the distribution of the LLR under low signal conditions is correctly modeled. We compare the LLR with other commonly used rejection algorithms such as the elliptical or sum of squared error tests. As a demonstration, we show the improved resolution in reconstructed super-resolution images when using the LLR rejection algorithm as compared with those generated using other rejection algorithms.

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Dynamics of Nuclear Protein Exploration Revealed by Intracellular Single Particle Tracking PALM

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Cellular regulation of eukaryotic cells involves molecular interactions of factors diffusing within the cellular volume. Understanding the gene expression regulation requires thus elucidating the spatio-temporal dynamics of intranuclear proteins at the single molecule level. However, live cell imaging of single molecules in eukaryotic cells has remained mostly restricted to processes occurring in the plasma membrane, readily accessible by TIRF microscopy as opposed to intra-nuclear processes.

We report an intracellular single particle tracking method using photoactivated localization microscopy that enables the study of protein dynamics inside live eukaryotic cells. So far single particle tracking PALM (sptPALM) (Manley et al, 2008) has been restricted to cellular systems for which imaging can be performed using total internal reflection microscopy (TIRF), and believed to be limited to slow diffusing systems (~ 0.1 um2/s). Here we demonstrate an approach that reduces the background of out-of-focus fluorophores by a tight control of the photoactivation, thus allowing the detection and characterization of single protein dynamics directly in the nucleus of living cells.

Applying this method to several nuclear proteins, we captured a wide range of diffusive behaviors from very rapid diffusion (> 10 μm2/s) to bound chromatin associated states (< 0.1 μm2/s). We measured the single molecule dynamics for a diverse set of proteins, from free fluorophores (Dendra2) with no known interactions in the nucleoplasm, to DNA binding (c-Myc), RNA binding (Fibrillarin), and protein-protein interacting complexes (p-TEFb). We observe that, overall, nuclear exploration is not governed by a unique nucleoplasmic geometry but rather a protein-specific variable. Our approach provides a versatile tool for single molecule in vivo studies in eukaryotes.

References

Manley, S. et al. High-density mapping of single-molecule trajectories with photoactivated localization microscopy. Nature Methods 5, 155–157 (2008).

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New Tool for Single Molecule Manipulation: Optical Pushing

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