enforcement a consistent PSF response through large depths, therefore permitting robust 3D-super-resolution volumetric imaging of fluorescence stained whole-cell samples or tissues. We show that our method allows imaging through 30 µm brain sections from the mouse frontal cortex by reconstructing fibrillar amyloid-β plaques found in Alzheimer's disease.

1398-Pos

3D Microscopy-Independent Approach for Obtaining 3D Super-Resolution Information in Rotationally Symmetric Bio-Structures

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Currently, it is highly desirable but still challenging to obtain high-resolution (<50 nm) three-dimensional (3D) super-resolution information of structures in fixed specimens as well as for dynamic processes in live cells. Here we introduce a simple approach, without using 3D super-resolution microscopy or real-time 3D particle tracking, to estimate 3D sub-diffraction-limited structural or dynamic information in rotationally symmetric bio-structures. This is a post-localization analysis that transforms 2D super-resolution images or 2D single-molecule localization distributions into their corresponding 3D spatial probability distributions based on prior known structural knowledge. This analysis is ideal in cases where the ultrastructure of a cellular structure is known but the sub-structural localization of a particular (usually mobile) protein is not. The method has been successfully applied to achieve 3D structural and functional sub-diffraction-limited information for 25-300 nm subcellular organelles that meet the rotational symmetry requirement, such as nuclear pore complex, primary cilium and microtubule. Herein, we will provide comprehensive analyses of this method by using experimental data and computational simulations.

1399-Pos

Restricting Diffusive Exchange *In Vitro* Demonstrates Inos Modulates Hypoxic Gradients in the Tumor Microenvironment

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The diffusion of metabolites in tissues produces gradients and heterogeneous microenvironments. As tumors grow, they eventually are no longer fully oxygenated by diffusion from normal vasculature. This leads to hypoxic regions within the tumor microenvironment that affect cellular processes and signaling pathways. Alterations in these pathways can lead to events such as angiogenesis and metastasis. We utilized a chamber system for cell culture that forms cell-generated hypoxic and metabolic gradients in two-dimensions by restricting the diffusive exchange of oxygen and metabolites to a monolayer of cells in a small volume – analogous to diffusion between a capillary and nearby tissue. Triple-negative mouse breast cancer cells (4T1s) were cultured in the chambers and live-cell imaging was used to quantify the evolution and extent of the oxygen concentration gradient. Cells migrated along the hypoxic gradient and formed a millimeter-scale disk of viable cells that was stable for at least one month. We quantified the spatial distribution of biomarkers such as HIF-1α, EMT markers, and proteins involved in inflammation by multiplexed, large-area immunofluorescence microscopy. The distribution of these markers correlated with the oxygen gradient. With this model, we studied changes in cellular signaling pathways as a function of cell position within the hypoxic gradient. Macrophages stimulated with lipopolysaccharide (LPS), an inflammatory cytokine, upregulated inducible nitric oxide synthase (iNOS), and nitric oxide (NO) production has been linked to decreased oxygen consumption in hypoxic environments *in vitro*. We investigated the interaction between iNOS in activated ANA-1 macrophages and hypoxia and demonstrated that treatment with LPS increased iNOS expression and altered the magnitude and spatial extent of hypoxic gradients. Funded by NCI Contract No. HHSN261200800001E.

1400-Pos

Protein Dimerization Probed with Site-Specific Attached Single Nanoparticles

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Self-association of proteins forming dimers or oligomers is a common occurrence in biological systems. Probing the dimerization status can be performed by various size- and mass-selective methods (e.g. analytical ultracentrifugation, gel filtration, ···). Every method has its strengths but generally the detection directly in the solution by optical means is preferred. Metal nanoparticles (NP) can be observed in a light microscope and their surface can be functionalized with different chemical compounds, like dyes or affinity tags. For example, nickel-chelated N-nitrilo-triacetic acid (Ni-NTA) functionalized metal nanoparticles (Ni-NTA NP) can be site-specifically attached to His-tagged proteins, forming a thin single protein layer on a NP. Additional functionalization of the Ni-NTA NP with Raman or fluorescent dyes provide furthermore the ability to observe red-shifted emissions, like surface-enhanced Raman scattering (SERS) or surface-enhanced fluorescence (SEF), especially if plasmonic metals are used as NP material. Those effects are large if single NPs are near to each other upon laser irradiation. In case the NP surface attached proteins form stable dimers or oligomers, the functionalized NPs will be concomitantly linked and in close proximity to each other, forming NP dimers or oligomers itself. The new formed NP-complexes can be detected by dynamic light scattering (DLS), asymmetric flow field fractionation (AFFF) or observed optically on the single-particle level by red-shifted emissions upon laser irradiation. Observing the red-shifted emissions, inelastic scattering (Raman/Mie) or fluorescence, concomitantly with elastic (Rayleigh) emissions via two separate imaging channels in a light-sheet illumination approach allows the check of the NP-dimerization/oligomerization status. This indicates protein interaction in the native suspension environment and enables determination of the ratio of non-oligomerized to oligomerized protein in a microscope image or video. This approach paves the way for fast optical determination of protein-protein or protein-ligand interactions.

Posters: EPR and NMR: Spectroscopy and Imaging

1401-Pos

Confidence Analysis of DEER Data and its Structural Interpretation with Ensemble-Biased Metadynamics

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Given its ability to measure multicomponent distance distributions between electron-spin probes, Double Electron-Electron Resonance spectroscopy (DEER) has become a leading technique to assess the structural dynamics of biomolecules. Recently, we have published two new methods for advancing the rigorous interpretation of DEER data (https://doi.org/10.1016/j.bpj.2018.08.008). First, building upon a model-based approach in which the distance probability distribution is represented as a sum of Gaussians or other functions, we use propagation of errors to calculate an associated confidence band for the distance distribution. This approach considers all sources of uncertainty, including the experimental noise, the uncertainty in the fitted background signal, and the limited time-span of the data collection. The resulting confidence band reveals the most and least reliable features of the probability distribution, thereby informing the structural interpretation of DEER experiments. Second, to facilitate the interpretation of distance distributions obtained from DEER experiments, we generalized the molecular-simulation method known as Ensemble-Biased Metadynamics. This method, originally designed to generate maximum-entropy structural ensembles consistent with one or more probability distributions, has been modified to account for the uncertainty in those target distributions as dictated by their confidence bands. After careful benchmarks, the proposed techniques have been demonstrated using DEER results from spin-labeled T4 lysozyme.
This work is now being extended to allow the determination of confidence bands for distance distributions obtained from the global analysis of multiple data sets.
https://lab.vanderbilt.edu/hustedt-lab/software/
https://github.com/Colvars/colvars

1402-Pos

Electron Paramagnetic Resonance Elucidates the Structural Mechanism by Which SERCA is Activated by DWORF

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We have used electron paramagnetic resonance (EPR) spectroscopy to examine the protein-protein interaction of a recently discovered 34-codon micropeptide,