Title

Analysis of membrane fluidity disturbances by mutant huntingtin in different membranal compartments using phasor analysis of local ICS and spectral phasor analysis

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surface. Several different immobilisation techniques have been tested for *N. gonorrhoeae* including polydopamine, Cell-Tak™, amino groups and polyclonal antibodies. Polydopamine is found to give the highest surface coverage, without significantly affecting the SCFI-measured fluctuation values. The use of antibodies also results in high surface coverage on the glass surface, however a larger amount of bacteria-to-bacteria binding is observed alongside slightly higher fluctuation values. We hypothesise that this increase is due to the allowance for a small degree of whole cell movement when attached with antibodies, an effect that is undesirable in this context due to the observation of sub-cellular motion. The results presented here confirm that, when operating at room temperature, the SCFI technique can be applied to *N. gonorrhoeae*, obtaining similar (however lower) fluctuation results to *E. coli.* As the viability of *N. gonorrhoeae* declines at room temperature, a specialised heating system has been designed and implemented, allowing measurements to be taken at 37°C.

675-Pos

Acquisition of cadherin order during desmosome assembly

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Desmosomes are structurally and functionally complex intercellular adhesive junctions that provide mechanical integrity to tissues by linking the intermediate filaments of neighboring cells. The basis by which desmosomes are able to resist mechanical stress, while also participating in dynamic tissue remodeling during wound healing and morphogenesis remains poorly understood. Desmosomal cadherins are Ca^{2+}-dependent transmembrane proteins that form the adhesive interface by binding to cadherins from neighboring cells. Electron microscopy has shown that cadherins are ordered, but not much is known about when or how this order is acquired. While the details of desmosome architecture are inaccessible by traditional, diffraction-limited optical microscopy, cutting-edge techniques such as Fluorescence Polarization Microscopy (FPM) can elucidate cadherin order. Fluorescently tagged proteins are expressed in cells that form desmosomes and imaged at varying excitation polarizations. The modulation of intensity as a function of the polarization angle corresponds to the net alignment of cadherins. Here, we synchronized desmosome assembly with a no Ca^{2+} pulse, which induces disassembly of all desmosomes. Reintroduction of Ca^{2+}, and subsequent monitoring of desmosomal cadherin desmoglein 2 (Dsg2) order over time, sheds insight into the relationship between cadherin order and adhesive function. Desmosomes completely disassemble after the low Ca^{2+} pulse; however, upon reintroduction of Ca^{2+}-containing media, we observe an increase in order that returns to WT levels by 8 h. Going forward, we will carry out dispase fragmentation assays which will be compared to the order time course and colocalization with adherens junction protein E-cadherin, which has been shown to help facilitate desmosome assembly. This will elucidate the relationship between cadherin order and desmosomal adhesive strength during desmosome assembly and maturation.

676-Pos

Analysis of membrane fluidity disturbances by mutant huntingtin in different membranal compartments using phasor analysis of local ICS and spectral phasor analysis

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Huntington's Disease (HD), caused by the expression of extended polyQ repeats in the protein huntingtin, is known to disturb cholesterol metabolism. The misfolded proteins alter biosynthesis and intracellular levels of cholesterol, leading to membranes deterioration. In this work, we aim to describe the effect of said disturbances in various membrane compartments caused by different polyQ expansions from the membrane fluidity point of view, an approach never applied before. To this end, we use the environment-sensitive fluorescent probe LAURDAN to study the membrane fluidity at different compartments of SHSY5Y cells modified to express the non-pathogenic (18QmCherry) and pathogenic (53QmCherry, 77QmCherry) protein. We focus on three categories of compartments: cellular plasma membrane, internal membranes, and lipid droplets, all known to be rich in cholesterol and other lipids. Our approach leverages LAURDAN's propensity to insert in hydrophobic regions, such as membranes, where its emission is the more blue-shifted the less water is accessible in the vicinity of the dye. A decrease in water content highly correlates with decreased membrane fluidity (bluer emission) and vice versa, allowing us to spatially map the membrane fluidity and lipid composition in living cells. For assessing fluidity in the membrane domains, we use the Spectral Phasor analysis where we analyze LAURDAN fluorescence to further quantify changes in membrane fluidity. Furthermore, to quantify the lipid droplets morphological properties, we use the Phasor analysis of Local Image Correlation Spectroscopy (PLICS), a technique capable of mapping the heterogeneity of spatial correlation functions. At the same time, we collect the fluorescence by mCherry-tagged polyQ aggregates to quantify their number and size. Together, these biophysical properties provide a direct visualization and quantitative approach in accessing membrane dysfunction in living cells.

677-Pos

A lateral resolution metric for static SMLM images from time-resolved pair correlation functions

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Single molecule localization microscopy (SMLM) permits the visualization of cellular structures an order of magnitude smaller than the diffraction limit of visible light, and an accurate, objective evaluation of the resolution of an SMLM dataset is an essential aspect of the image processing and analysis pipeline. Prior work has employed pair auto-correlation functions to directly measure the effective spread of localizations arising from single labeled objects, providing a method for measuring the lateral resolution of an image. Here we incorporate an explicit temporal dependence into this approach, calculating full space-time auto-correlation functions to quantify resolution as a function of the time lapse between localizations. In doing so we find that the past approach reports primarily on localization precision since correlation functions are often dominated by contributions from multiple localizations of the same fluorophore at short time intervals, especially when single molecule blinking kinetics resembles (d)STORM. Examining resolution at longer time-intervals reports on other factors that limit resolution, such as sample drift or imperfect drift correction. This resolution metric reports on how precisely one can measure pairwise distances between labeled objects and is complementary to the commonly used Fourier ring correlation (FRC) metric that also considers spatial sampling. The method is demonstrated on simulated localizations, DNA origami rulers, and antibody labeled cellular structures.

678-Pos

Optically accessible microfluidic flow channels for non-invasive high-resolution biofilm imaging using lattice light sheet microscopy

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An imaging system that can achieve long-term, high-resolution imaging of biofilms is essential for studying cellular level dynamics within bacterial biofilms. Combining high spatial-temporal resolution and low phototoxicity, lattice light sheet microscopy (LLSM) has proven powerful for non-invasive 3D imaging of living cells and tissues. However, its application in biofilm research is limited because the open-on-top imaging geometry using water-immersion objective lenses is not compatible with living bacterial specimens; bacterial growth in the media basin and on the microscope's objective lenses makes long-term time-lapse imaging impossible. To make LLSM compatible with long term imaging of live bacterial specimens, we have developed hermetically-sealed, but optically-accessible, microfluidic flow channels. A thin polymer film was glued across 3D-printed channel, where the top channel wall has been omitted, to generate liquid- and gas-tight channel. Negligible optical aberrations were achieved by using polymer films that precisely match the refractive index of water. Since bacteria do not adhere to the polymer film itself, the polymer window provides unobstructed optical access to the channel interior. Inside the flow channels, biofilms can be grown on arbitrary, even non-transparent, surfaces. Using this flow channel device in the LLSM, we were able to record growth of *S. oneidensis* MR-1 biofilms over several days at cellular resolution without any observable photodamage. Hermetically-sealed, optically-accessible, microfluidic devices, combined with non-invasive LLSM, thus enable long duration cellular level imaging of bacterial biofilms under precisely controllable physical and chemical conditions. These capabilities open the door to the study of emergent properties of microbial populations in terms of the fully-resolved behavioral phenotypes of individual cells under a tunable, near-native environment.