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Investigation of breast cancer metabolic plasticity in the tumor microenvironment by single-cell organelle phenotyping

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The Antisecretory Factor (AF) is an endogenous protein that inhibits intestinal hypersecretion and various inflammation disorders *in vivo*. AF has been detected in many mammalian tissues and plasma, but its mechanisms of action are essentially unknown. Previously, we studied the pharmacological action of the AF on $GABA_A$ receptors in cerebellar granule cells, comparing the electrophysiological response evoked by two-photon mediated release of caged GABA compounds before and after the administration of AF-16, a 16 amino acids long peptide obtained from the amino-terminal end of the AF protein. After the treatment with AF-16, we observed an increase in the $GABA_A$ receptor responses, particularly in those containing the α_6 subunit. To figure out the interactions of AF with $GABA_A$ receptors in the same cellular model (cerebellar granule cells), we combined immunofluorescence subunits' staining with confocal and super-resolution microscopy. In particular, we took advantage of an innovative imaging technique that combines stimulated emission depletion (STED) with fluorescence lifetime microscopy (FLIM) to collect super-resolution 3D maps of different subunits distributed on the neuron cell membrane. We explored different approaches to analyze super-resolution fluorescence images obtained by labeling α_1 and α_6 subunits before and after 1-hour incubation with AF-16. Comparing pre- and post-treatment maps, we found differences in how different subunit populations respond to AF treatment. We critically analyzed these new experimental findings with our previous electrophysiological data to widen the knowledge of the mechanisms of interaction between $GABA_A$ receptor subunits and AF protein.

641-Pos
Quantitative image reconstruction for microscopy with correct sub-pixel photon statistics

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The diffraction of light forces a lower limit on spatial resolution available with conventional optical microscopes. However, sophisticated computational techniques in conjunction with inhomogeneous illumination have facilitated image acquisition without this limitation. Typical image reconstruction algorithms for such superresolution microscopy techniques, including structured illumination microscopy (SIM), involve combining multiple diffraction-limited raw images in Fourier space via minimization of some cost function. This leads to undesired artifacts including loss of correct noise properties for the photons collected by the camera and loss of temporal resolution. Here, we propose a Bayesian framework performing reconstructions in real space while incorporating all sources of noise such as camera electronics and incoming photon Poisson statistics. Furthermore, this framework inherently allows corrections for inhomogeneous illumination incident on a biological sample. As a result, superresolution reconstructions can now be performed with fewer images otherwise needed by existing reconstruction methods while maintaining Poisson statistics for collected photons at the sub-pixel level. We benchmark our framework using both simulated and experimentally generated images.

642-Pos
Investigation of breast cancer metabolic plasticity in the tumor microenvironment by single-cell organelle phenotyping

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Metabolic plasticity, i.e., the capability of cells to modify their metabolic state, is a hallmark of cancer and an important factor in the formation of metastases as well as a major contributor to chemoresistance and tumor recurrence. Furthermore, the tumor microenvironment, namely the collection of environmental properties (pH, nutrient availability, fatty acids) and cell types that surround the main tumor mass, is regarded to as the main driver of the metabolic state of tumor cells. Unfortunately, no technique can non-invasively assess the metabolic state of single cells in living samples, limiting our understanding of the heterogeneity and dynamics of metabolic state transitions. Here, we apply a technique, named ESPRESSO (Environmental Sensors Profiling Relayed by Subcellular Structures and Organelles) that combines organelle-specific environment-sensitive probes (ESPs) with hyperspectral imaging and quantitative bioimage analysis. We use a mixture of lipid droplet-, mitochondria- and lysosome-specific ESPs to quantify of morphological (e.g., number, size, organization) and functional (e.g., membrane potential, pH, polarity) characteristics to identify the metabolic state in single, living cells. To understand the differences in metabolic plasticity of non-tumorigenic breast epithelial (MCF10a) and triple negative breast cancer (MDA-MB-231) cells, we determined their metabolic state with ESPRESSO under a variety of stress and environmental conditions, identifying the characteristic metabolic signature of the cell lines under those conditions. We further investigated the response to diverse chemotherapy drugs (doxorubicin, paclitaxel, curcumin) in an effort to understand their effect on cancer and healthy cells, highlighting the environmental conditions that would favor the resistance to a specific drug treatment. Taken together, we provide a framework to identify the metabolic response of cancer and non-tumorigenic cells under different drugs, stressors and environmental conditions, providing in-depth characterization of their metabolic response at the single cell level.

643-Pos
Estimating the localization spread function of static single-molecule localization microscopy images

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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 data set is an essential aspect of the image processing and analysis pipeline. Here, we present a simple method to estimate the localization spread function (LSF) of a static SMLM data set directly from acquired localizations, exploiting the correlated dynamics of individual emitters and properties of the pair autocorrelation function evaluated in both time and space. The method is demonstrated on simulated localizations, DNA origami rulers, and cellular structures labeled by dye-conjugated antibodies, DNA-PAINT, or fluorescent fusion proteins. We show that experimentally obtained images have LSFs that are broader than expected from the localization precision alone, due to additional uncertainty accrued when localizing molecules imaged over time.

644-Pos
Optical sectioning of label-free samples using standard bright-field microscopy

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The three-dimensional (3D) images produced by the traditional bright-field (BF) light microscope are ill-defined and therefore require reconstruction. Current alternatives to perform 3D BF imaging require dedicated instrumentation or extensive computer modeling. We report optical sectioning in bright-field microscopy (OSBM), a direct method for 3D imaging of label-free samples, where the BF inverse-imaging problem is solved in real space by using an elemental combination of hardware and software. Our method consists in acquiring z-stack images of minimally treated samples under Köhler illumination in the coherent regime, followed by a digital image processing pipeline designed to localize the source of axial intensity gradients that correspond to the z-locations of scatterers within the sample. We validate OSBM by visualizing fungal, animal tissue, and plant samples and comparing with light sheet fluorescence microscopy imaging, finding excellent match for sparse spatial distributions. Our work demonstrates how the standard microscope can be used as an effective 3D imaging device.

645-Pos
Construction and characterization of a versatile multimodal single-molecule super-resolution microscope for 3D whole cell studies

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