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### Title

Spatiotemporal single-cell phenotyping in living 3D skin organoids

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hydrogel matrix with the charged surfaces of proteins. Currently, we are working on characterizing protein diffusion in a more complex extracellular matrix (ECM) using fcsSOFI.

#### 626-Pos

##### Metabolic profiling of transferred mitochondria using FLIM intensity based image segmentation (FIBIS)

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Bulk metabolic assays on cell populations have shown that mitochondria transfer trigger breast cancer cells turn towards oxidative phosphorylation (OXPHOS) in favor of proliferation, migration, and cell growth. Yet, the current methods lack single cell resolution of interaction between endogenous and exogenous mitochondria to help understand the consequence metabolic alterations and other cell fate decisions. The phasor approach to fluorescence lifetime imaging microscopy (FLIM) has been widely used to measure the free to bound fraction of reduced form of NADH to quantify metabolic changes in live cells but not at the single mitochondrial scale. Here, we developed the FIBIS algorithm as a robust approach to recognize mitochondria from NADH intensity and further analyze mitochondrial metabolic states to investigate mitochondria transfer. We demonstrate that the NADH autofluorescence perfectly localizes with Mito7-mRuby labeled mitochondria and is free from artifactual emission spectral overlaps. Our data indicates that there was a 40% and a 20% increased fraction of bound NADH after mitochondria transfer in MCF7 and MB231 cells, respectively. This suggests that there is an enhancement of OXPHOS for both breast cancer cells. This was in correlation with the results from the Seahorse XF analyzer where the oxygen consumption rate was significantly higher after uptake of isolated mitochondria from breast epithelial cells. We also used Mitometer to show that there were increasing numbers of mitochondria and branching which imply that the fusion of exogenous and endogenous mitochondria are highly adaptable and increase cellular respiration. In summary, the FIBIS enables single mitochondrial metabolic profiling and aids in understanding the effects of transferred mitochondria to recipient cells. Our results also indicate that transferred mitochondria in cancer cells enhances OXPHOS, increased branching, and mitochondrial numbers, thereby decreasing cell viability and increasing sensitivity to chemotherapy agents.

#### 627-Pos

##### Single-molecule orientation-localization microscopy resolves the nanoscale organization of self-assembled peptides

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Self-assembling peptides have remarkable applications in biomaterials and therapeutics. Amphipathic peptides with alternating polar and nonpolar residues tend to self-assemble into cross- $\beta$ -rich fibrils, which are usually signatures of various protein-misfolding pathologies. Key to understanding the mechanisms of self-assembly is the ability to visualize and quantify the complex, heterogeneous organization of these peptides with single-aggregate sensitivity. Here, we utilize single-molecule orientation-localization microscopy (SMOLM), a variant of super-resolution microscopy, to measure amphipathic KFE8 (Ac-FKFEFKFE-NH<sub>2</sub>) assemblies. Using the transient binding of Nile red (NR) as a blinking mechanism, we find that the orientation distribution of NR on KFE8 is significantly different from that bound to amyloid-beta fibrils, where NR is simply oriented parallel to the coverslip and along the long axis of each fiber. A detailed analysis of NR orientations on KFE8 shows that they bind in a manner consistent with a helical ribbon model of the underlying peptide assembly. Further, the 3D orientations of NR enable us to quantify the pitch-to-diameter ratio of the underlying helix. Finally, we show that SMOLM can resolve a variety of nanoscale structures formed by model self-assembling peptides. Due to limited spatial resolution, standard localization-based super-resolution microscopy cannot resolve these details. These experiments are the first demonstration of utilizing fluorophore orientation "spectra", i.e., the 3D orientations of dye molecules, to resolve the complex nanoscale organization of self-assembled peptides in solution.

#### 628-Pos

##### Spatiotemporal single-cell phenotyping in living 3D skin organoids

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Continuous monitoring of cell states in living tissues remains elusive despite the explosion of single cell technologies. Here, we use organelle-specific environment-sensitive probes (ESPs) combined with hyperspectral imaging and a dedicated quantitative analysis pipeline to spatially and temporally track keratinocyte cell states in living 3D skin organoids. Our technique, named ESPRESSO (Environmental Sensors Profiling Relayed by Subcellular Structures and Organelles) combines hyperspectral imaging and phasor un-mixing, enabling imaging of up to 6 ESPs (targeting chromatin, mitochondria, lysosomes, tubulin, Golgi apparatus and lipid droplets) at the same time with a single laser excitation. The quantification of morphological (e.g., number, size, organization) and functional (e.g., membrane potential, pH, polarity) characteristics of the organelles and subcellular structures allows us to identify the cell state at the single-cell level and is applicable to living cells in 2D and 3D. Cell state and cell-cell interactions are tightly regulated in space and time to determine tissue function. However, the spatiotemporal regulation of cell states and their interactions at the systems level during skin development and dysfunction remains poorly understood. Determination of cell states is routinely achieved by high-dimensional methods such as single-cell RNA sequencing (scRNA-seq), which yields the transcriptional profile at the single cell level of cell cultures and tissue. However, processing of the sample is required with consequent loss of spatial information. Genetic modification to include fluorescent tagging of a biomarker of interest can sometimes be used for living samples, although this approach is limited to observing a few parameters at one time and it may not be applicable to primary cells due to difficulty in transfection and early loss of proliferative capacity. Here, we demonstrate the capability of ESPRESSO to characterize keratinocyte differentiation as a function of space and time in living 3D skin organoid.

#### 629-Pos

##### Visualizing both nucleosome disassembly and reassembly during FACT chaperone activity

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The histone chaperone FACT (FACilitates Chromatin Transcription) enhances transcription in eukaryotic cells, targeting DNA-protein interactions. FACT, a heterodimer in humans, comprises SPT16 and SSRP1 subunits. We measure nucleosome stability and dynamics in the presence of FACT and critical component domains. Optical tweezers quantify FACT/subdomain binding to nucleosomes, displacing the outer wrap of DNA, disrupting direct DNA-histone (strong site) interactions, altering the energy landscape of unwrapping and increasing the kinetics of DNA-histone disruption. Atomic force microscopy reveals nucleosome remodeling and DNA unwinding. Single molecule multi-channel fluorescence observes component histones as they disassemble under tension and are lost. Yet if tension is released, reassembly is also observed and surprisingly both loss and reassembly are accelerated by key domains of FACT. We resolve this ambiguity, noting that all histone-DNA kinetics are faster with FACT. Of the individual domains we studied, two were shown to influence nucleosome structure and dynamics with distinctly contradictory functions; while the SSRP1 HMGB domain *displaces* DNA, SPT16 MD/CTD *stabilizes* DNA-H2A/H2B dimer interactions. However, only intact FACT tethers disrupted DNA to the histones, and supports rapid nucleosome reformation over several cycles of force disruption/release. These results demonstrate how key FACT domains combine to catalyze nucleosome disassembly and reassembly.

#### 630-Pos

##### Towards an unbiased shape classification of super-resolution microscopy localizations

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Single molecule localization microscopy (SMLM) captures biological structures at nanoscale spatial resolution and has become an important tool for discovery in cell biology in recent years. However, tools that enable robust quantification of complex biological structures have lagged behind. In this