dependent kinase 1 (Cdk1) inhibitors had similar effects on the FLIM signature, suggesting that Cdk1 acts on Complex I; this was confirmed using oxygen consumption measurements to directly measure flux through Complex I, as well as by direct detection of Complex I phosphorylation at Cdk1 consensus sites. Four different optical reporters were then used to quantify the effect of Complex I activation on the dynamic oscillations in β-cell NAD(P)H, mitochondrial membrane potential ($\Delta\Psi m$), ATP/ADP, and cytosolic Ca^{2+}. The glucose-dependence of each of these parameters, assessed by oscillatory plateau fraction, was correspondingly reduced by Cdk1 blockade. We conclude that the Cdk1-Complex I pathway is activated as a compensatory mechanism to meet the increased metabolic demands placed on pancreatic β-cells in obese animals, and that untargeted NAD(P)H-FLIM fingerprinting has the potential to identify precise molecular changes in the pancreatic islet metabolic pathways.

2398-Pos Board B542

Time-Resolved Study of Triplebody-Mediated Lysis by Natural Killer Cells on Microstructured Target Cell Arrays

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Natural Killer (NK) cells are reduced in numbers and cytolytic activity in bone marrow and peripheral blood of Acute Myeloid Leukemia (AML) patients at diagnosis, but generally recover during chemotherapy. Certain antibody-derived therapeutic agents, such as single chain triplebodies, depend on the availability of sufficient numbers of active NK-cells. To monitor recovery of these cells during chemotherapy, sensitive new methods are needed, capable of quantitating key properties, while using only small numbers of primary cells. A new method (single cell cytometry) is presented herein, which uses arrays of isolated target cells to study the ability of triplebody 33-16-123 to recruit Natural Killer (NK) cells. The single cell cytometry (SCC) approach measures the fraction of cells killed by NK cells in an automated high-throughput fashion using time-lapse fluorescence microscopy. We evaluate the fraction of specifically lysed cells, representing the incremental lysis in the presence of the triplebody, mediated by NK cells both as a function of triplebody dose and the effector-to-target cell (ET) ratio. The specifically lysed fractions are in agreement with standard assays. We observed a systematic dependence of killing rates with time, indicating an enhancement and saturation of NK activity at high doses. The results demonstrate the potential of cell arrays for time-resolved studies of immune effector cells interacting with cancer cells and therefore elucidate the role of antibody-derived agents on effector cell activation.

2399-Pos Board B543

Dynamic Optical Displacement Spectroscopy to Quantify Biomembrane Bending Fluctuations

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Stochastic bending fluctuations of biomembranes are of high physiological importance, e.g., for preventing adhesion between red blood cells or for the establishment of cell substrate contacts in cell adhesion. Unfortunately, especially in nucleated cells they are notoriously difficult to quantify. To meet this challenge we developed a new technique based on Fluorescence Correlation Spectroscopy (FCS). We use comparatively high labeling of the biomembrane (~10000 fluorophores in the focal spot) to suppress all contributions of lateral diffusion to fluctuations of the fluorescence intensity. Under these conditions all intensity variations arise from axial motion of the membrane. Best results are achieved when the focus is placed slightly above or below the membrane. The resolution, as estimated for ideal membranes is 20 nm spatial and 10 μs temporal.

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Single-Particle Tracking Analysis using the Radius of Gyration Tensor, Revisited

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A key problem in the analysis of trajectories from two-dimensional single-particle tracking experiments is distinguishing actual structure due to anomalous subdiffusion, confinement, or directed motion from apparent structure due to fluctuations in random walks. To better analyze these trajectories we examine properties of descriptors based on the radius of gyration tensor of the trajectory. This work revisits and updates my work [Saxton, Biophys J 64 (1993) 1776] and work from the Amblard laboratory [Coscoy et al., Bull Math Biol 69 (2007) 2467]. The descriptors are chosen to be the time-scaled eigenvalues of the radius of gyration tensor. Both individual and joint probability density functions of the descriptors are considered, using analytical results from the literature and Monte Carlo simulations. Particular attention is paid to the time scaling of the histograms so one can easily obtain the histogram for a given number of experimental time steps.The current view in the physics literature on single-particle tracking is that the analysis should be done entirely in terms of the displacement over single time steps, $\Delta t = 1$. This approach is clearly appropriate for pure random walks. These are Markovian, and displacements for $\Delta t > 1$ by definition cannot add any information. But for non-Markovian motion, $\Delta t > 1$ is directly relevant, as the method of Thiel et al. [PRL 111 (2013) 010601] to identify the mechanism of anomalous diffusion by scaling analysis of displacements over pairs of Δt's. Biophysically interesting forms of non-Markovian motion include directed motion, which is positively correlated; anomalous subdiffusion, which is anticorrelated on all time scales; and confined motion, which is anticorrelated on the time scales of individual collisions with the corral walls and diffusive crossing of the corral.
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Quantitative Determination of Phototoxicity in Live Cell Super-Resolution Microscopy

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In the past decades a number of novel fluorescence super-resolution microscopy techniques have emerged allowing to obtain images of cell structures and dynamics with unprecedented resolution by overcoming the diffraction limit. More recently, these approaches have been applied to imaging dynamics in living cells. Cell structures and molecules are in these applications labeled with cell permeable fluorescent organic dyes (e.g SiR, Oregon Green) or endogenously expressed fluorescent proteins. Since super-resolution microscopy tends to require higher laser radiation, phototoxicity and photodamage are potential limitations in live-cell fluorescence microscopy.

Here, we present our recent systematic investigation of phototoxicity and photodamage in STED and FPALM/PALM/STORM super-resolution microscopy using a number of different cellular stress indicators, Ca^{2+} release (through measuring fluorescence levels of Fluoforte), Mitochondria depolarization (through measuring the decrease in fluorescence of JC-1) and reactive oxygen species formation (through monitoring the increase in fluorescence of MitoSOX, CMFDA and Enzo Total ROS/Superoxide indicator). We focus, in particular, on short to mid-term effects (up to 30 minutes), which is a typical time-scale of many cell-biological processes (membrane trafficking, etc.). We investigated, the influence of intensity, fluence, wavelength, dye location in the cell (e.g. ER, Golgi, mitochondria, cytoplasm using SNAP tags) and dependence on dye/tag labeling ratio.

2402-Pos Board B546

Illuminating Dynamic Processes in the Embryogenesis of Caenorhabditis Elegans with Lighsheet Microscopy

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Understanding dynamic processes in cells and multicellular systems requires spatiotemporally resolved imaging techniques with low photobleaching and -toxicity. Following this demand, single-plane illumination microscopes (SPIMs) have recently emerged as powerful tools. Using a custom-made SPIM setup that combines rapid widefield detection with optical sectioning and reduced bleaching, we have performed long-term, three-dimensional in-vivo imaging on early embryos of the small nematode Caenorhabditis elegans [1]. Based on our SPIM data, we were able to quantify the movement and arrangment of cells during early embryogenesis. The remarkable similarity of individual cell trajectories across different embryos is well captured by a simple model that mainly considers mechanical interactions of cells [1].

Going beyond mere imaging, we have also implemented a SPIM-based fluorescence correlation spectroscopy (FCS) approach, which allows for quantifying protein diffusion in the early embryo at high spatiotemporal resolution. As a first application, we have determined spatially resolved diffusion maps of the peripheral membrane protein PLC1δ1 in the cytoplasm and on the plasma membrane of worm embryos in the one- and two-cell stage. The obtained results compare favorably to previous reports on the same protein construct. We also have determined time-resolved diffusion maps of the protein PIE-1 which highlight a significantly varying mobility of the protein along the anterior-posterior axis of the embryo before the first, asymmetric cell division.