multiphoton/confocal microscope. A source of photoactivated PAGFP was generated within selected compartments using multiphoton excitation at 820nm and its dissipation was monitored with 488nm confocal scanning. Images were compared with the output of a 3D diffusion model to estimate effective radial and axial diffusion coefficients.
Results: PAGFP diffusion in the IS was isotropic and faster than in the OS, $D_{IS} = 5.2\ \mu m^2\ s^{-1}$. In the OS PAGFP diffusion was anisotropic, with faster radial diffusion, $D_{OS\text{-}radial} = 3.5\ \mu m^2\ s^{-1}$, and slower axial diffusion, $D_{OS\text{-}axial} = 0.19\ \mu m^2\ s^{-1}$.
Conclusion: PAGFP diffusion in both compartments was substantially retarded relative to aqueous solution, $D_{aq,PAGFP} \sim 90\ \mu m^2\ s^{-1}$, and cultured Chinese hamster ovary (CHO) cell cytoplasm, $D_{CHO,PAGFP} \sim 20\ \mu m^2\ s^{-1}$. Moreover, axial diffusion of PAGFP in the OS was hindered to a larger extent than expected from the geometry of disc membranes that span the compartment orthogonal to the cylinder axis. These results suggest that the photoreceptor cytoplasm possess higher density of cytoskeleton and/or macromolecules.

2055-Pos
Lipopolysaccharide Regulation of Dendritic Cells Activation and Life Cycle: in vitro and in vivo Studies Towards Antitumor Immunoactivity
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Dendritic cells (DCs) are key regulators of innate and adaptive immune responses that can be exploited in the immunological treatment of many type of cancers. Recently, we have demonstrated that lipopolysaccharide (LPS) is able to regulate DC life cycle through the activation of a CD14-dependet pathway [1]. Once activated with LPS, DCs become also able to prime Natural Killer (NK) cells to exert their anti-tumoral activity as demonstrated both *in-vitro* and *in-vivo* using mouse models in which melanoma tumors were sub-cute implanted. Using two-photon microscopy, we are currently extending these experiments to the direct investigation of the interaction of LPS-activated DCs with NK cells in *in vivo* condition at the level of peripheral lymph nodes.
DCs and NK cells,labeled with different fluorescent markers, are tracked continuously in the lymph nodes while the structure of the lymph node is monitored by second harmonic generation microscopy. The analysis of the traces and the comparison of the experimental results to statistical and simulative models of the lymphocytes motion allows to elucidate their dynamic behavior at different times after the activation of the DCs shedding new light on the DCs - NK cells interaction.
[1] Zanoni, I.; Ostuni, R.; Capuano, G.; Collini, M.; Caccia, M.; Ronchi, A.E.;Rocchetti, M.; Mingozzi, F.; Foti, M.; Chirico, G.; Costa, B.;Zaza, A.; Ricciardi-Castagnoli, P.; Granucci, F. (2009) CD14 regulates the dendritic cell life cycle after LPS exposure through NFAT activation. ***Nature,*** 460:264-269.

2056-Pos
Growing Lung A549 Epithelial Cells on Metallic Surfaces
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Fluorescence can be greatly enhanced near metal surfaces due to many-fold increased brightness and photostability of fluorophores located near metallic nanoparticles or nanostructures. Further more, fluorophores deposited on a plane of a 50 nm thick silver or gold mirrors, show directional fluorescence in a form of hollow cone. These favorable properties of fluorophore-plasmonic interaction can be utilized in high-sensitivity imaging of cellular processes. However, the cell growth strongly depends on the nature of the substrate and is often very difficult on bare metal surfaces. In our study we examined suitability of different metal surface coatings for growing lung A549 epithelial cells. Six different surfaces were tested - glass, silver mirror, silver mirror coated with SiO_2, gold mirror, gold mirror coated with SiO_2 and silver fractals on glass. The glass coverslips with five different metallic surface coatings and one control were placed in a tissue culture plates containing DMEM-High Glucose media. Suspension of $0.5x10^6$ cells/plate was deposited on the slides and the cell cultures were placed in 37°C, 5%CO_2 incubator. Cell growth was monitored every 24 hours. No substantial differences in cell morphology were found whether they were on the regular microscopic glass slide or slide covered with metals, but cells initially grew significantly slower on fractals compared to other "smooth" surfaces. The findings demonstrate feasibility of growing A549 cells on metal-coated glass surfaces and opens the opportunity for imaging live A549 cells using metal enhanced fluorescence.
Supported by the Canadian Institutes of Health Research and the Canadian Cystic Fibrosis Foundation (R.G), and Emerging Technologies Fund Grant Texas (Z.G.).

2057-Pos
Fluorinated Voltage Sensitive Dyes for SHG and Multiphoton Microscopy
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Dyes based on hemicyanine chromophores have high membrane affinities and the ability to report on local membrane environment and act as sensors of membrane voltage. Laser-scanning second harmonic generation (SHG) microscopy utilizing such voltage sensitive dyes has shown considerable promise as an imaging modality, possessing several advantages over fluorescence for the optical mapping of membrane potentials. The addition of electronegative fluorine atoms to the chromophore is intended to lower both the ground and excited state energies, so as to make dyes less susceptible to photobleaching. Improved photostability will allow extension of the duration of optical recording measurements, permit the use of more intense laser excitation, and minimize photodamage to the biological sample. The effect of fluorination on photostability and dye performance has been systematically investigated for a series of newly synthesized dyes and found to depend critically on the location of the substituent within the chromophore. Voltage-clamped neuroblastoma cells stained with these dyes were imaged with 1064nm excitation, allowing sensitivities and response kinetics of SHG and two-photon fluorescence to be determined simultaneously for several fluorinated dyes. Our results suggest that voltage sensitive dyes can be developed which have large SHG signal changes, sufficient photostability, and the requisite speed for use as a practical tool for measuring electrical activity in biological systems.
(Supported by NIH grant EB001963)

2058-Pos
High Throughput Screening of Biosensor Domains: Visualizing Dynamic Activation of Src Kinases in Live Cells
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Biosensors that report activation of native, unmodified proteins can help delineate complex cellular processes with minimal perturbation of normal behavior. However, sensors for endogenous proteins are rare, in part due to the absence of readily available binding reagents that are selective for the active form of the targeted molecule. To address this issue we combined high throughput screening of engineered state-specific binding elements with a fluorescence-based reporting system that turns these elements into biosensors. We have targeted Src family kinases (SFKs) since they are key signaling nodes that control numerous cellular functions, including migration and adhesion. Also, the multiple roles performed by Src kinases and their involvement in several signaling networks suggest spatio-temporally regulated pools of SFK activities. Phage display screening was used to generate fibronectin domain III (FN3) binders that selectively bind SH3 domains from Src family kinases. Pull down experiments demonstrated that an FN3 binds selectively to active Src kinases. Using merocyanine dyes developed in our lab for live cell imaging, we have converted this FN3 into a sensitive fluorescence-based biosensor for activation of Src family kinases. The new sensors reveals patterns of Src activation in migrating cells and in cells stimulated with growth factors. In migrating cells, a distinct band of Src activation was observed at the leading edge. This transient activation coincided with protrusion. We also observed precisely timed Src activation in linear and circular dorsal ruffles. In keeping with our overall aim of developing a generally applicable strategy of sensor design, we have generated multiple binders using HT screening and are developing biosensors specific to individual Src family kinases.