goal was to create a system for screening libraries of randomly mutagenized fluorescent proteins for better 2P properties. We created an optical system for collecting 1- and 2-photon induced fluorescence images from entire petri dishes of E. coli colonies. Libraries of randomly mutagenized eGFP were screened for improved 2P/1P fluorescence ratios. Screening the first round of evolution showed a slightly larger ratio spread outside the range of parent eGFP. One hundred of these clones were subjected to a second round of screening. Colonies that showed reproducible 2P/1P ratios greater than the parent eGFP were selected to move forward. A selective pool of the best 18 clones (library A), and a more inclusive pool of the best 59 clones (library B), were used for a new round of gene shuffling and random mutagenesis. After only two rounds of evolution, libraries A and B contained at least 5 distinct mutant populations with significantly increased 2P/1P ratios. Sequence analysis of 96 clones from these libraries revealed that every clone harbored at least one mutation. Eleven unique mutants were used for a third round of evolution. Screening and sequence analysis of the third library, reveals thirteen mutations that either individually (V68M, S27G, V163A, T203I) or in combination (E6G, T65S, Q80R, N105S, D1117G, N121S, Q184R, S202N, V219I) significantly shift the 2P/1P fluorescence ratios.

4093-Pos Board B281
Modular Design of a Tandem Dye Tunes the Photophysical Properties of a Biosensor
Matharishwar Naganabau,1 Saumya Saurabh,2 Marcel Bruchez2.
1Department of Chemistry, Carnegie Mellon University, Pittsburgh, PA, USA, 2Department of Chemistry, Biological Sciences, Molecular Biosensors and Imaging Centre, Carnegie Mellon University, Pittsburgh, PA, USA.
Fluorogenic Activating Protein Extracts (FAPs) are protein reporters which harvest the fluorescence of a non-covalently bound dark molecule (fluorogen). Light-harvesting dendrimers consisting of multiple chromophores have been built to improve the brightness of a biosensor. The multiple donor dyes transfer energy to the lone acceptor, thus increasing the brightness of the biosensor. Although bright, these light harvesting structures (dyedroms) displayed reduced association constants with the cognate protein, and reduced photostability of the fluorogen-FAP complex. To understand and potentially overcome these limitations, we have synthesized Cy3-Malachite Green based donor-acceptor structures with linker lengths from 10 to 30 atoms and found that longer linker lengths correspond to reduced quantum yield, but not reduced energy transfer efficiency, and that this arises from protein-dye changes that are slow relative to protein-dye association. Ensemble and single molecule level investigation of triplet state quenchers (TSQs) covalently linked to the fluorogen and the tandem dyes provide deeper insights into the development of bright, photostable probes for imaging applications.

Micro- and Nanotechnology III

4094-Pos Board B822
Chemical Cell to Cell Communication on Biomineraled Nanopore Substrates for Single Cell Analysis
Abhishek J. Dharan1, Kai-Chun Lin2, Patty Senechal-Willis1, Laimonas Kelbauskas3, Deirdre Meldrum1, Michael Goryll2.
1Department of Chemistry, Carnegie Mellon University, Pittsburgh, PA, USA, 2Department of Chemistry, Biological Sciences, Molecular Biosensors and Imaging Centre, Carnegie Mellon University, Pittsburgh, PA, USA, 3Biodesign Institute, Arizona State University, Tempe, AZ, USA. A method for chemically stimulating selected cells and observing cell to cell chemical communication on a biomineraled nanopore substrate provides an enabling tool for researchers studying diseases at the single cell level to better understand interactions between cells. By developing a method to analyze chemical communication between cells at the single cell level rather than determining behavior of large cultures, researchers can accurately pinpoint the responses cells have to various chemical stimuli. The biggest challenge in developing a method for observing intercellular chemical communication is determining the optimal methods for collecting quality data on living cells as they interact with one another.

The goal of this research project is to determine how cells can grow on biomineraled nanopore substrates that are mounted on silicon micropore chips, allowing selected cells to be chemically stimulated so that cell to cell chemical communication can be observed.

Results will be shown on the fabrication of biomineraled nanopore substrates and on preliminary experiments showing cell growth factors that determine the ideal conditions for cell growth on the biomineraled nanopore substrates. Several different characterization methods will be employed, ranging from microscopic observation to optical assays like fluorescence spectroscopy of chemically stimulated cells. Results will be analyzed using statistical methods used in previously published studies looking at cell viability on substrates.

4095-Pos Board B283
High-Throughput Screening of T Cell Cytotoxic Events by Biomass Profiling
Thomas A. Zangle, Daina Burnes, Colleen Mathis, Owen N. Witte, Michael A. Teitell.
UCLA, Los Angeles, CA, USA.
Adaptive immunotherapies against cancer, in which cytotoxic, CD8+ T cells engineered to express T cell receptors (TCRs) targeting cancer-associated antigens are transplanted into a patient, have shown dramatic promise in clinical trials. A major impediment to the widespread use of this technique for treatment of diverse cancers is the lack of a fast approach for the identification of TCRs from patient samples. In this talk, we present a method for high-throughput screening of T cell/target cell interactions by measurements of cell biomass. This live cell approach is label-free and allows cells to be recovered for downstream analysis. To ensure specificity, three parameters are tracked: target cell appearance, target cell mass loss during cell death, and T cell mass during and after the cytotoxic event (Figure panels a-c). Our results demonstrate, for the first time, the kinetics of T cell mass increase during activation. Finally, we will present an extension of this method to a micro-fabricated microwell format for the screening of patient samples and discovery of novel TCRs.

4096-Pos Board B824
Parallel Magnetic Tweezers for Pulling CNS Axons Towards a Source of Repellent Factors
Gil Lee, Devrim Kilinc, Agata Blasiak.
School of Chemistry and Chemical Biology, University College Dublin, Dublin, Ireland.
Regeneration of central nervous system (CNS) axons into hostile environments such as the glial scar that forms after the spinal cord injury is a crucial medical problem. Successful therapies will combine pharmaceutical interventions with physical techniques that control the mechanical environment along the axon path. Axon growth cones are highly motile regions that constantly probe the surrounding tissue and integrate external chemotactic stimuli into motility decisions such as elongation, retraction or turning. While a range of chemical guidance molecules and subsequent signaling pathways are well established, the effect of mechanics on axon elongation is largely unknown. We describe a novel in vitro system that enables direct force application to a large number of CNS growth cones that are fluidically isolated from their cell bodies and exposed to controlled gradients of diffusing guidance cues. Growth cones were preferentially targeted by anti-neuronal cell adhesion molecule (NCAM) magnetic particles. Particles exhibited low non-specific interaction with axon shafts and resulting in a massively parallel magnetic tweezers platform. Results suggested that pulling with <10pN force can occasionally accelerate growth only if rho-associated protein kinase (ROCK) is inhibited. We show that up to 30pN force can be applied to a single magnetic particle. In separate experiments, a transient gradient of the repulsive guidance molecule Semaphorin 3A was formed in the axonal compartment. Our results suggest that developing CNS axons are not slowed down but deviated away from the source in a dose-dependent manner. This deviation was blocked by inhibiting ROCK or Calpain suggesting a multiplexed control over the growth cone cytoskeleton. Our current work aims to dissect the roles of Rho and Calpain pathways in the motility of growth cones, which are pulled towards a source of Sema3A.

4097-Pos Board B825
Biological Compatibility of Electromanipulation Media
Anthony J. Asmar1,2, Ahmet C. Sabuncu1,4, Mark A. Levenstein1,5, Michael W. Stacey1, Ali Beskoc.
1Frank Reidy Research Center for Bioelectrics, Old Dominion University, Norfolk, VA, USA, 2Biological Sciences Department, Old Dominion University, Norfolk, VA, USA, 3Mechanical Engineering, Istanbul Technical University, Istanbul, Turkey, 4Institute of Micro&Nanotechnology, Old Dominion University, Norfolk, VA, USA, 5Mechanical and Aerospace Engineering, Old Dominion University, Norfolk, VA, USA, 6Mechanical Engineering, Southern Methodist University, Dallas, VA, USA.
Electromanipulation of cells, which includes dielectrophoresis, electrorotation, and physical cell manipulation and characterization tool has gained particular interest recently. However, the applicability of electromanipulation to biological cells is limited to cells suspended in special types of media containing lower amounts of salts in comparison to physiological buffers. Impedance measurements of cells are often performed in low conductive buffers, mainly due to parasitic capacitance that occurs at the electrode
electrolyte interface. One might question the use of electromanipulation buffers as it results in lower transmembrane transport and possible increased cellular death. Therefore, in this study, effects of electromanipulation buffers with varying salt concentrations on cellular viability and transmembrane transport was studied for a human T-cell leukemia cell line (Jurkat). Cellular viability was measured in vitro using an MTT cell viability assay. Cellular death was also measured using the trypan blue dye exclusion method. Membrane capacitance and conductance was measured by impedance spectroscopy over a range of extracellular salt concentrations. We describe the cell membrane conductance at different extracellular salt concentrations using a quantitative model. Even though membrane conductance measurements by impedance spectroscopy is indicative of number and activity of ion channels even performed at low extracellular salt levels; results indicate that cells in electromanipulation buffers have reduced viability. Strategies to increase metabolic activity by changing buffer composition while still retaining the performance of impedance spectroscopy and electromanipulation are also presented.

4098-Pos Board B826
Inherently Fluorescent Nanowires for Cellular Mechanosensing
Karl Adlerhoff, Henrik Persson, Zhen Li, Stina Oredsson, Udo Häcker, Magnus T. Borgström, Christelle N. Prinz.
University of Lund, Lund, Sweden.

How mechanical cues trigger cellular events is currently an intensive research area and measuring the forces involved requires multiple and highly sensitive probes. Arrays of vertical nanowires have been shown to measure cellular forces down to 15 pN, providing there is a high precision in the determination of the nanowire deflection. Current approaches involve the use of either organic functionalization of the nanowires, or scanning electron microscopy (SEM) to determine the deflection of the nanowires. However, organic fluorophores are prone to photo-bleaching and SEM can only measure the forces at a given time point and may also give rise to artifacts due to cell shrinkage during the dehydration step.

Our approach is to use epifluorescence, vertical, gallium phosphide (GaP) nanowires with a top-segment made of gallium indium phosphide (GaInP), which is inherently fluorescent. The nanowires are grown using metal-organic-vapor-phase epitaxy (MOVPE), which allows for excellent control over nanowire diameter, length and material composition. GaInP is strongly fluorescent and photo-stable, which enable the precise determination of the nanowire tips for a long period of time.


4099-Pos Board B827
CMOS Electrochemical Sensing Platform for Spatially Resolved Detection of Redox-Active Metabolites Released by Multicellular Films
Daniel L. Bellin1, Hassan Sakhtah2, Jacob K. Rosenstein3, Peter M. Levine4, Jordan Thimot1, Kevin Emmett5, Lars E.P. Dietrich2, Daniel L. Bellin.
1Department of Electrical Engineering, Columbia University, New York, NY, USA, 2Department of Biomedical Engineering, Columbia University, New York, NY, USA, 3School of Engineering, Brown University, Providence, RI, USA, 4Department of Electrical and Computer Engineering, University of Waterloo, Waterloo, ON, Canada, 5Department of Physics, Columbia University, New York, NY, USA. Departments of Electrical Engineering and Biomedical Engineering, Columbia University, New York, NY, USA.

Despite advances in monitoring spatiotemporal expression patterns of genes and proteins with fluorescent probes, direct detection of metabolites and small molecules remains challenging. Metabolite detection is of particular interest in microbial biosignals. Mass spectroscopy (MS) techniques have been applied to metabolite detection in biofilms, but the instrumentation is bulky and expensive. Additionally, MS techniques focus on probing biofilm top surfaces, which requires various forms of colony treatment such as liquid bridges, imprinting on a substrate, coating with an organic matrix, or incorporation of tags. Scanning electrochemical microscopy (SECM) can be used to study electrochemically active metabolites on the top surface of biofilms in a spatially resolved fashion, but without the ability to quantify concentration or to simultaneously detect multiple redox-active species. Here, we demonstrate spatially resolved detection and quantification of metabolites released from imaging electron microscopy by interfacing biofilms to a complementary metal-oxide-semiconductor (CMOS) integrated circuit (IC). The chip contains an array of working electrodes and parallel potentiostat channels. "Images" over a 3.25-mm-by-0.9-mm area can be captured with a spatial resolution of 750 µm limited currently by diffusion through the thin (1-mm) agar film interfacing the biofilm with the chip. Using this platform, we demonstrate that square wave voltammetry (SWV) can be used to detect, identify, and quantify (for concentrations as low as 2.6 µM) four distinct phenazines, redox-active metabolites produced by Pseudomonas aeruginosa PA14. We characterize phenazine production in both wild-type and mutant P. aeruginosa PA14 colony biofilms, and find correlations with fluorescent reporter imaging of phenazine gene expression. Key to the methods developed in this work is the exploitation of IC technology as a new tool for biology, enabling many simultaneous measurement channels and electrodes to be fabricated within a very small area.

4101-Pos Board B828
An Integrated Liver- and Heart-On-A-Chip Platform
Bioengineering, UC Berkeley, Berkeley, CA, USA.

Engineering microfluidic devices to mimic human organs’ microenvironments addresses many of the challenges of in vivo models, such as the lack of controllability and repeatability, and conventional static in vitro models, including deficiency in mimicking organ complexities. Due to these advantages, ”organ-on-a-chip” platforms have gained significant interest for drug screening and studying the biophysics of physiologically relevant microenvironments. Here, we present an integrated heart- and liver-on-a-chip platform by culturing human induced pluripotent stem cells-derived cardiac myocytes (hiPSC-CMs) and hepatocytes (hiPSC-HPs) in a single microfluidic chip. Since the failure of most of drugs is regarded to the toxicity or dysfunction of these two organs, simultaneous and integrated investigation of the biocompatibility of the liver and heart is essential. A dynamic fluid transport system was engineered by creating endothelial-like barriers to mimic continuous nutrient exchange and cell-cell interaction observed in ventricular myocardium and liver sinusoid. Moreover, using microfluidic channels, the blood flow between liver and heart was mimicked. Laminar fluid flow in these microchannels provides a predictable and spatially and temporally controllable microenvironment to study the biophysics for these two organs on chips. This model not only gives us the ability for testing the effects of heart diseases’ drugs on the functionality of the cardiac and liver cells simultaneously, but it also presents the role of detoxification on the heart performance. The effect of different parameters, such as flow rate, was investigated by real time monitoring of the cultured cells morphology and metabolism. The capability of our device for long-term culturing of iPSC-derived liver and cardiac cells was also presented, which is critical for patient-specific drug discovery. We believe that this integrated organ-on-a-chip platform provides new possibilities for the future of drug screening and personalized medicine.

4104-Pos Board B829
High-Throughput Single-Cell Analysis Device for Following Simultaneous Intracellular Signaling Events
Amin A. Banaeiyan1,2, Daryneh Ahadmopour1, Caroline B. Adels1, Mattias Gokso¨r.
1Bioengineering, UC Berkeley, Berkeley, CA, USA, 2Physics, University of Gothenburg, Gothenburg, Sweden.

Investigating the behavior of single cells in a high-throughput manner calls for the design and realization of innovative, robust and versatile devices capable of conducting adaptable experiments to address the desired biological questions. For instance, the possibility of running experiments that compare the reaction of different cell types to a specific stimulus or the effect of a given substance at different concentrations on a specific cell type is highly desirable. One inherent challenge associated with running such experiments, however, is the existence of random uncertainty factors from experiment to experiment. Examples of this could be common measurement errors in preparing exact substance concentrations or the likeness of consecutive cell cultures.

Here, we report the design and fabrication of a high-throughput microfluidic platform intended for parallel investigations on single cells upon extracellular environmental changes. We fabricated a hydrodynamic-based cell isolation microfluidic block in polydimethylsiloxane (PDMS) with two secluded entities. The comb-shaped trapping zone in each entity consists of 52 V-formed individual traps. Each trap has the dimensions of 10 µm × 10 µm and connects two complement microchannels via a 2-µm wide confinement opening. After the cell-loading step, isolated cells can undergo several chemical-rinsing steps and, in combination with fluorescence microscopy imaging, cellular response can be read and analyzed. This easy-to-operate, parallel microfluidic platform enables the simultaneous comparison of the read out between two portions of a particular cell passage to various chemical stresses or different concentrations of one specific stimulus.