The ability of bacteria to squeeze into constrictions a fraction of their size can lead to bacterial infections such as osteomyelitis. In osteomyelitis, bacteria cells squeeze into small channels in the bone where they are protected from immune cells and become much more difficult to treat with antibiotics.

To simulate the mechanical loading conditions bacteria experience as they move through sub-micron channels, bacteria are analyzed in similarly sized tapered channels through the fabrication of a microfluidic device. The sub-micron channels were created using Deep UV (DUV) Photolithography in the CNF, and then etched to transfer the design to a fused silica device. The design includes an inlet of approximately 1.2 µm, an outlet of 250 nm, and is 75 µm long. The bacteria trapped in the channels are imaged with both traditional and super-resolution microscopy. The distance travelled is then measured and compared to the pressure drop experienced by the bacteria in the channels. This pressure drop is defined as the difference in pressure across the bacteria trapped in the channel. Data collected this summer revealed that under a high inlet pressure, the bacteria can go into a channel width a fraction of their size. At a low inlet pressure, the bacteria will not squeeze into a constriction less than half their diameter. However, with the introduction of a large pressure drop, the bacteria can squeeze into a constriction up to a quarter of their diameter (Figure 1).

The device uses 600 different channels so that several different pressure drops can be examined at once. The silica glass device is created with DUV lithography. Fused silica was chosen because it is much stiffer than the bacteria and its transparency allows for microscopy. SEM, AFM, and the Profilometer are used to check for proper taper geometry, outlet width, channel depth and to allow optimization of the process in subsequent application.

After characterization, the device is bonded to a thin (170 nm) silica wafer and ready for experimentation. The device is placed on the microscope stage and bacteria in liquid suspension are flowed into the inlet using a syringe pump with a pressure gage reporting pressure at the inlet. Experiments are performed at
inlet pressures of 25 kPa and 60 kPa. Using a syringe pump that controls flow rate, the bacteria in M9 media are transported into the device. A pressure sensor is connected to the tube and a computer so that the pressure can be monitored and controlled based on the flow rate of the syringe pump. Images of the bacteria in the tapered channels are collected using transmission microscopy and the distance traveled by the bacteria was determined. The pressure drop across each bacterium in a tapered channel is determined using hydraulic circuit calculations. The distance traveled by bacteria is determined using the channel's number labels as a positional marker in the experimental images. The results indicate the relationship between loading conditions and how far the bacteria travel.

Results and Conclusions:
Over 1000 cells from over 20 different experiments were analyzed. Experiments were performed at both 60 kPa and 25 kPa inlet pressure. At a 60 kPa inlet pressure, the regression line shows an intercept of 52 µm traveled (75 µm channel length), and an average width of 500 µm under a 1 kPa pressure drop. \( R^2 = 0.39 \). At a 25 kPa inlet pressure, the regression line shows an intercept of an average of 30 µm traveled. \( R^2 = 0.71 \). In regards to reaching max constriction (250–300 nm) in the channels, this can be done in the 60 kPa experiments at approximately 6 kPa pressure drop, while it takes at least double that in the 25 kPa conditions. It was previously undetermined whether in the 25 kPa experiments the bacteria would be able to reach the end of the channels where it is under the max constriction. Therefore, these findings present the lab with new information regarding loading conditions that enable bacteria to squeeze deep into these channels (Figure 4).

Future Work:
Future work would be to conduct a similar experiment, but with bacteria known to cause infections, such as *Staphylococcus aureus*. Since this strain of bacteria has a higher safety level than the *E. coli* used, the study would have to be moved to a different lab facility. As more of a long-term goal, we want to find out what component or components of bacteria play the biggest role in how they are able to squeeze into these sub-micron channels. Finding the biological pathways in which bacteria squeeze into these sub-micron channels could eventually lead to better antibiotics for treating bacterial infections.

Acknowledgements:
NSF grant nos. ECCS-1542081 and CMMI-1463084, NNCl, CNF REU Program Coordinators and Staff. Thank you to Professor Christopher Hernandez and Melanie Roberts for affording me this opportunity, and the Peng Cheng Group for collaborating on this study.
Nanostamp Optimization for Single-Molecule DNA/Protein Array Studies

2017 CNF REU Intern: Mónica M. López Martínez
CNF REU Affiliation: Chemical Engineering, University of Puerto Rico-Mayaguez Campus

Abstract:
Over the past two decades, biophysical single molecule DNA, RNA and motor protein studies have demonstrated the important role that the structural and mechanical properties of single molecules play in gene replication and expression. Specifically, genetic processes can be significantly affected when DNA experiences torque or protein interaction forces, which happens often in vivo. Single molecule DNA studies typically “tether” DNA between a protein anchor on a surface, and a microbead in solution, that can be twisted or pulled with optical or magnetic tweezers. Nanostamping of protein spot anchor arrays, compared to blanket coating a substrate with protein for DNA anchoring, aids in single molecule studies by precisely controlling the DNA anchor position. Thus, we are exploring a nanostamping method called the “Ink-Subtract-Print” method by optimizing the dimensions of electron beam-patterned nanostamps that can selectively pattern arrays of 100-300 nm wide circles of protein on a glass surface.

Summary:
Introduction. The deoxyribonucleic acid molecule (DNA) has a double helix structure with unique mechanical properties that subsequently influence the way that other proteins interact with DNA to achieve complex biological functions of transcription and replication.

Our lab is interested in studying the interactions between DNA and motor proteins that are present in DNA processing activities. Although DNA can be macroscopically long, it is only 2 nm wide and cannot be visualized with light microscopy, DNA is therefore tethered between a microsphere handle and microscope slide for observation and manipulation. To obtain organized observations of these microscopic molecules and investigate interactions between a protein and multiple DNA strands, we precisely placed the position of the separate DNA molecules on a glass slide so that the distance between strands was well-defined. Specifically, we patterned a protein surface array using the Ink-Subtract-Print (ISP) nanostamping method [1], so the DNA could be anchored on the surface at one end of the DNA molecule with high spatial precision (Figure 1).

Methods. 1. Ink-Subtract-Print Method. This protein patterning procedure consists of inking a PDMS square with protein to cover the whole surface, and then stamping the square on the patterned silicon template to remove the protein everywhere on the PDMS except in the array of small circles [1]. This subtractive step is physically possible because plasma cleaning the silicon template gives hydrophilic properties to the wafer. Since the protein on the PDMS is also hydrophilic, it will be attracted to the top surface of the subtraction template wafer. When the PDMS is pulled away, the protein will be attached to the wafer in the non-patterned areas. In contrast, the PDMS will retain protein where the silicon wafer had holes. The patterned protein spots on the PDMS after subtraction are, then, stamped or “printed” on a plasma-cleaned glass surface which will be the area used to perform the DNA-binding experiments (Figure 2).
Methods; II. Nanofabrication of Patterned Silicon Template. Our main goal thus was to create patterned silicon templates containing periodic hexagonal arrays of 100-300 nm circular holes spaced at a 3-µm pitch that could be used as a subtraction template (Figure 3a) to produce patterned protein spots on PDMS (Figure 3b).

To acquire the target pattern needed to perform the Ink-Subtract-Print stamping procedure on our silicon wafer, the first step was to expose a silicon wafer coated with ZEP520A resist using the JEOL JBX-6300FS 100kV electron beam (e-beam) lithography system. Once the wafer was exposed and developed, the Oxford Cobra inductively coupled plasma (ICP) etcher was used to etch the silicon wafer according to the e-beam pattern. To remove the ZEP520A and obtain our final cleaned, patterned nanostamp template, we used a bath of 1165 organic stripper for approximately four hours. Finally, we obtained four 1 cm × 1 cm stamp areas patterned on each wafer.

Results:
After four hours of DNA incubation time (13.7 kbp DNA, 25 pM), we observed an average of 10 tethers in each microscope field of view, which contained ~ 1,500, 150 nm wide protein spots. To increase the number of tethers, the incubation time was increased to 17 hours and yielded ~ 50-100 DNA-protein tethers per field of view. By increasing anchor protein spots to 300 nm in diameter, we were able to obtain more tethers in a shorter amount of time compared to 150 nm wide protein spots. An analysis of these results shows that since the area of each protein anchor spot is small compared to anchor spots described in previously published reports [2], it will take more time for the DNA to bind to these small features (Figure 4).

Future Work:
There are several ways to improve the number of DNA tethers in the future work: (1) Increase DNA concentration (2) tether DNA to magnetic beads and place a magnet under the glass so the DNA is more likely to dwell near the cover slide surface and thus more likely to bind to the protein anchor or (3) test larger stamp circles.

Acknowledgements:
NSF and NNCI funding via NSF grant no. ECCS-1542081, Cornell NanoScale Facility, PI Dr. Michelle Wang, Mentors Ryan Badman and Jim Baker, Lab Members Jaeyoon Lee, Guillermo V. Vargas, and Seong Ha Park, CNF REU Program Coordinator Melanie-Claire Mallison, CNF Staff Michael Skvarla, Tom Pennell, Jeremy Clark, and Edward Camacho.

References:

Multi-Organ Microphysiological Systems for Drug Screening

CNF Project Number: 731-98
Principal Investigators: Michael L. Shuler, Harold G. Craighead
Users: Ying Wang, Paula Miller, Chen-yu Chen

Affiliations: Nancy E. and Peter C. Meinig School of Biomedical Engineering, Robert Frederick Smith School of Chemical and Biomolecular Engineering; Cornell University
Primary Sources of Research Funding: National Center for Advancing Translational Sciences, National Science Foundation, National Institutes of Health
Contact: MLS50@cornell.edu, hgc1@cornell.edu, ying.wang@cornell.edu, pgm6@cornell.edu, cc2569@cornell.edu
Website: https://www.bme.cornell.edu/people/profile.cfm?netid=mls50
Primary CNF Tools Used: VersaLaser laser cutter, Objet30 Pro 3D printer, PDS 2010 Labcoter2parylene coater

Abstract:

Novel preclinical screening models that can better predict clinical outcomes are in urgent need to expedite drug development at sustainable cost. Human cell culture-based, multi-compartmental multi-cellular microphysiological systems (MPS) hold the potential of recreating the dynamic in vivo drug process and human cell response in vitro and are thus promising to serve as a “human surrogate” for drug screening. These microphysiological systems are featured with microfluidic interconnections among chambers that mimic the blood circulation in the body allowing for organ-organ interactions. We are currently developing several multi-organ MPS, with different emphases on lung drug inhalation, spleen immune responses and central nervous system (CNS) drug uptake.

Summary of Research:

Lung-on-a-Chip Platform for Inhalation Drug Screening and Development. Pulmonary diseases such as asthma and chronic obstructive pulmonary disease (COPD) are the leading causes of respiratory death. Current preclinical drug screening process for inhalation drugs for treatment relies on animal models and is often costly and time consuming. Drug administration through inhalation for long-term sustained release has also gained substantial recognition in recent years. The development of Lung-on-a-Chip models aims to bypass animal models and provide a high adaptive way to fit into the human anatomy. We have developed a pumpless microfluidic inhalation drug screening system that could be used to evaluate drug inhalation, metabolism and toxicity. This microphysiological system includes a “breathing” lung chamber, a liver and a tumor chamber with 3D tissue constructs (Figure 1). The lung chamber was designed to mimic human alveolar-capillary interface and active air flow breath function. The expansion of the lung chamber was driven by vacuum pump via an expandable silicone membrane (Figure 1). The Lung-on-a-Chip platform consists of two poly(methyl methacrylate) (PMMA) layers and two silicone layers, which were patterned using a CO₂ laser cutter VersaLaser at CNF. The microfluidic channels and reservoirs were etched to desired dimensions into the bottom PMMA layer using the CO₂ laser.

Liver-BBB-Tumor Chip. We are developing a three-organ human microphysiological system that could be used for central nervous system (CNS) drug screening. The device contains three cell chambers, representing “liver,” “blood brain barrier (BBB),” and “tumor,” respectively. The organ chambers are interconnected via microfluidic channels and perfused with blood surrogate at physiologically relevant flow rates using a

Figure 1: Design of the lung-on-a-chip device.
pumpless microfluidic platform [1,2]. The microfluidic platforms were fabricated using a 3D object printer (Objet 30Pro at CNF) and coated with a conformal layer of parylene-C via a vapor deposition system (PDS-2010 Lab LABCOTER® 2 at CNF) for enhanced chemical resistance and biocompatibility. The BBB constructs were prepared with brain microvascular endothelial cells (BMECs) derived from human induced pluripotent stem cells (hiPSCs) and primary human astrocytes that were co-cultured on the two sides of a porous polycarbonate membrane [3]. Liver 3D cultures were prepared from human primary hepatocytes and non-parenchymal cells (NPCs) encapsulated in hydrogels. The tumor chamber was filled with hydrogel encapsulated MCF-7 cells. Our Liver-BBB-tumor model could be used to evaluate drug metabolism by liver cells, brain uptake of drug and its metabolites across the BBB and the toxicity towards tumor cells. This model will be a valuable tool for screening of brain drug candidates.

**Design and Demonstration of a Five-Compartment Microphysiological System.** We describe a human “Body-on-a-Chip” device (or microphysiological system) that can be used to emulate the in vivo drug process of absorption, distribution, metabolism and toxicity as well as immune responses. The device contains five different chambers representing bone marrow, inflamed spleen, GI tract, liver and fat (Figure 2). The design of the 5-organ MPS is based a physiologically based pharmacokinetic (PBPK) model.

The barrier chamber layer (GI tract) allows direct access for administration of chemical or biological compounds, which must pass through the GI barrier and undergo a first-pass hepatic metabolism before entering the circulation. The non-barrier chambers were created as three-dimensional configurations by encapsulating cells in PGMatrix (Peggel, Manhattan, KS). The reported average human male organ volumes and organ flow rates [4] were used to estimate the fluid retention times in each organ chamber. A custom programmed rocker platform was used to create circulation using gravity-drive flows. The silicone cell culture chamber layers and plastic channel layers were patterned using the CO₂ laser VersaLaser at CNF. The device is optically transparent and thus allows for daily monitoring. This five-chamber device will be used to study preclinical anti-leishmaniasis drug toxicity and response.

**References:**


Improved Single Cell Whole Genome via Microfluidic Platform

CNF Project Number: 762-99
Principal Investigator: Harold G. Craighead
Users: Harvey C. Tian, Sarah Reinholt

Affiliation: Applied and Engineering Physics, Cornell University, Ithaca
Primary Source of Research Funding: National Cancer Institute
Contact: hgc1@cornell.edu, hct33@cornell.edu, sjr236@cornell.edu
Primary CNF Tools Used: ABM contact aligner, Unaxis deep silicon etcher

Abstract:
We present a valveless microfluidic device for single cell capture and on-chip whole genome amplification of immobilized deoxyribonucleic acid (DNA). This device uses a series of closely spaced micropillars to physically entrap single cells and its genomic DNA (gDNA) upon cell lysis. Our past work has demonstrated high efficiency DNA capture using micropillar arrays from varying cell counts (hundreds) down to a single cell [1]. Here, we demonstrate the ability to perform in-channel chemistries, such as DNA amplification, to the immobilized gDNA in our device by sequentially introducing reagents into our channels. We describe a method for using our device to reduce amplification bias during isothermal whole genome amplification (WGA) of single cells. We report that out of six gene loci sampled, we detect all six loci from the single cell WGA product collected from our device compared to two out of six in control samples. Single cell WGA has long suffered from amplification bias. To this end, several technologies have been developed to improve genome coverage and reduce amplification bias including emulsion based techniques [2], linear amplification [3], and microfabricated devices [4,5]. Our approach fundamentally differs from existing methods as we perform WGA under a constant flow of reagents across extracted gDNA that has been elongated and immobilized within our device.

Summary of Research:
Our device, as shown in Figure 1(A), contains a single input port leading to multiple separate yet identical channels each containing a micropillar array region, Figure 1(B). A suspension of human cervical cancer cells (HeLa cells) are loaded into the input single cells were captured at the apex of the micropillar array as depicted in Figure 1(B) while excess uncaptured cells flow through to the output ports where they were removed. Upon introducing a cell lysis agent into the channel, the gDNA of entrapped cell become physically entangled on the micropillars immediately downstream from the original position of the cell and this gDNA can be labeled and visualized as in Figure 1(C).

The extracted gDNA can then be isothermally whole genome amplified on-chip using commercially available multiple displacement amplification (MDA) reagents. Because the amplified DNA fragments are below the size threshold necessary to become entangled upon the 2 µm diameter micropillars, they flow through the pillars and can be collected at the output port at the end of the amplification.

Figure 1: Microfluidic device schematic and micrograph of entrapped single cell and extracted DNA. The fabrication of this device was done using the CNF ABM contact aligner and Unaxis deep silicon etcher.
To assess amplification bias, we sampled six cancer gene loci spaced across the human genome. We used PCR and targeted primer pairs to determine the presence or absence of each loci within the WGA product. Positive controls were performed on single cells isolated through fluorescence activated cell sorting (FACS).

Our results indicate that single cell genomes amplified with our device showed 6/6 gene loci detected as compared to 2/6 from samples in which single cells isolated via FACS. We believe the ability to perform amplification and other processes on immobilized but accessible DNA in a flowing system is responsible for the reduced amplification bias and provides a system for other processes on single cell genomes.

References:
Graphene Transistors for Biological Sensing

CNF Project Number: 900-00
Principal Investigator: Paul L. McEuen
Users: Michael F. Reynolds, Samantha Norris, Kathryn L. McGill

Abstract:
Two-dimensional materials provide a platform for biocompatible yet conformal cellular sensing devices. We report a releasable graphene field-effect transistor fabricated using standard photolithography, and we demonstrate the electrical response of these devices to action potentials from cardiomyocytes and mouse neurons in vitro.

Summary of Research:
Presently, there are numerous methods for single-cell action potential detection, but all struggle to simultaneously obtain high spatial, temporal, and electrical resolution. The use of moveable microscopic graphene field-effect transistors (FETs) has the potential to solve many of these problems. Since graphene has remarkable voltage sensitivity, the action potential passing through a nearby cell can be easily detected by measuring the current flowing through the device. Moreover, freely-released graphene devices can be manipulated to conform to the cells of interest [1], thereby maximizing the signal recorded in this extracellular measurement. Finally, graphene absorbs only 2% of white light. Its effective transparency allows electrical measurements to be performed simultaneously with numerous imaging techniques, granting high spatial resolution coincident to an electrical measurement of cell action potentials.

We pattern the graphene using standard photolithography techniques. This allows us to play with designs to allow for a more conformal placement on the cell without degrading the electrical responsivity of the device [1]. To prepare these samples, we initially cover our substrate with 20 nm of aluminum oxide (alumina) grown via atomic layer deposition (Oxford ALD), as shown in Figure 1.1. This layer of alumina is a sacrificial layer and will be etched away shortly before the experiment (see Figure 1.3). After transferring graphene, grown via chemical vapor deposition, on top of the substrates, we evaporate 50 nm of gold on top of these samples and pattern it using standard photolithographic processes (exposing with the Autostep i-line Stepper). As shown in Figure 1.2, the final photolithography step is to oxygen plasma etch the graphene into the desired shape (Oxford 81).

After etching away the alumina in hydrochloric acid, the devices, now weakly adhered to the substrate, are placed into the same Petri® dish in which the neural or cardiac cells were cultured. In order to manipulate the devices, platinum-iridium probes were attached to micromanipulators; by forcing these probes into the gold pads, sufficient contact is made to pick up the device. To reduce electrical noise but still allow current to flow, the shafts of the probes were insulated except for a small exposed tip. It is important to note that from the time the alumina is etched away, the devices must remain in fluid; otherwise, we are unable to remove them from the surface and freely manipulate them in space. After picking up a device, it is then placed upon a cell. Experiments are performed in standard cell media,
and the electrolyte potential of the media is controlled by a Ag/AgCl reference electrode inserted into the fluid.

An experiment with graphene devices placed on top of cardiomyocytes is shown in Figure 2. As expected, the graphene appears as a mostly transparent sheet stretched between the two gold pads. To monitor the current through the graphene device, a small voltage bias of ~100 mV is placed across the device by connecting one of the probes to a battery. We monitor the current flowing through the device using an amplifier connected to either an oscilloscope or data acquisition hardware, depending on our experimental needs.

Successful experiments have been performed with non-released devices (upon which cells are grown) using cardiomyocytes at Cornell and with releasable (free-floating) devices on mouse neurons by collaborators at Oregon State University. The devices used at Cornell were fabricated at CNF, and the cardiomyocytes were cultured by Tyler Kirby, a postdoctoral fellow in the Lammerding lab at Cornell. The resulting data is shown in Figure 3. The neuronal data agrees with the literature [2], yet the cardiomyocyte action potential spike is ~100x wider and smaller than anticipated.

Moving forward, we plan to improve our graphene devices to increase the signal-to-noise ratio of our data and improve device-cell contact to capture more of this trans-membrane current. Presently, we are experimenting with device geometries and characterizing a process to put silicon dioxide o-rings between the graphene devices and cells. In doing so, we hope to trap the ions released by the cell during a firing event, thereby increasing the local voltage gating of the graphene. We will also be considering new methods for manipulating our devices with the ultimate goal of performing an electrical measurement in vivo.

References:
A MEMS Microtensiometer for Sensing Water Potential in Plants and Soils

CNF Project Number: 1119-03
Principal Investigator: Abraham D. Stroock
Users: Michael Santiago-Pinero, Winston L. Black II, Siyu Zhu, Olivier Vincent, Antoine Robin

Affiliation: Department of Chemical and Biomolecular Engineering, Cornell University
Primary Sources of Research Funding: National Science Foundation (CBET-0747993 and CHE-0924463), the Air Force Office of Scientific Research (FA9550-15-1-0052), the National Institute of Food and Agriculture, U.S. Department of Agriculture (under Agreement No. 2010-51181-21599)
Contact: abe.stroock@cornell.edu, ms2343@cornell.edu, wlb62@cornell.edu, sz393@cornell.edu, robin.antoine93@gmail.com
Website: www.stroockgroup.org
Primary CNF Tools Used: Thermal oxide furnace, LPCVD furnace, Oxford 81/82, SÜSS SB8e, Oxford PECVD, DISCO dicing saw

Introduction:

We have been pursuing the application of the second generation micro-tensiometer in environmental and agricultural contexts and for the study of related fundamental phenomena. A micro-tensiometer (µTM) measures the temperature and water potential (equivalent to the chemical potential of water) in its immediate environment for applications in physical chemical research on the properties of liquid water.

We have reported the design of the second generation µTM, and the preliminary results about the response of embedded sensors in trees last year. This year, we focused on applying the µTM for monitoring the plant water status, and the study of metastable solid-liquid equilibrium of water. We have developed the methodology to install a µTM into the trunk of a tree, and conduct diurnal stem water potential measurements; the observed values agree well with the benchmark, manual technique. We have also managed to provide the first direct measurement of the Gibb-Thomson equilibrium between ice and liquid in the doubly metastable state of tension and supercooling.

Summary of Research:

Motivation. This project seeks to develop a microelectromechanical system (MEMS) tensiometer capable of sensing water potential. Water potential, Ψ (MPa) is a thermodynamic quantity that defines the thermodynamic availability of water for chemical reactions and physical processes such as mass transfer. Of particular interest is the range of Ψ near saturation that occurs in the plants and soil that make up the biosphere. Accurate, in situ measurements in this range have not been achievable with current technologies.

The ability to perform such measurements has important implication for studying the basic biology of plants, for precision agricultural techniques such as deficit irrigation, and for basic questions about the thermodynamics of water itself.

Design. Figure 1 presents images of our micro-tensiometer. The top view shows the wiring associated with a strain gauge in the form of a Wheatstone bridge of poly(silicon) piezoresistors and a platinum resistance thermometer. The bottom view shows a cavity that we fill with pure liquid water; the layer of silicon above this cavity acts as a diaphragm, the deflection of which is measured by the strain gauge on the top side. This cavity is connected to the bottom edge via channels and a zone of nano-porous silicon that we form as a thin layer on the bottom side of the wafer; the design of this structure was inspired by the structure of xylem, the conductive tissue in plants. The cavity and microchannels are seal by bonding the bottom side of a glass wafer. As water leaves the cavity through the nanoporous membrane, the pressure of the liquid in the cavity drops until it comes to equilibrium with the external phase of water. We measure the difference in pressure between the inside and outside gives us Ψ and is measured with the calibrated strain gauge.
Method. Figure 2 presents an abbreviated representation of our process flow. The important steps in this process are: growth of insulating oxide (B2 thermal oxide furnace), deposition and patterning of poly(silicon) (LPCVD furnace C4), etching of cavity and microchannels (Oxford 81/82), anodic etching of porous silicon membrane, anodic bonding of silicon to glass (SÜSS SB8e), deposition and patterning of platinum with titanium adhesion layer, deposition of passivation layers (Oxford PECVD), and dicing. After dicing, chips were mounted and wire bonded to custom PCBs and selectively encapsulated in various materials to protect electronic elements from water and while allowing for exchange through the membrane.

Application in Plants. Figure 3 presents the comparison between the response of a µTM embedded directly within the stem of a potted apple tree (solid curve, right y-axis) and a Scholander pressure chamber (black circles, right y-axis). As expected, temperature (dashed curve, left y-axis) rises during the day and decreases at night, and the water potential decreases during the day after transpiration starts in response to sunrise, and increases when the transpiration slows down and the stem rehydrates with water feed from the soil. The measured stem water potential was within the range for a well-watered apple tree (> -15 bars), and matched the Scholander pressure chamber, a widely accepted hygrometer that requires point by point manual measurements and high-pressure gas.

Studies on Metastable Solid-Liquid Equilibrium of Water. Figure 4 presents the water potential of ice measured by direct submersion of a µTM inside ice and with cooling of the system down to -2.2°C. Figure 4A presents a schematic diagram of the direct contact between ice and metastable liquid water in the sensor. Figure 4B shows the linear correlation between the system temperature and measured water potential (MPa). The tension increases with the decreasing of temperature at the rate predicted by the Gibbs-Thomson relation (-1.2 MPa/°C).

Current Efforts:
We are building a mathematical model to predict the stem water potential in plants as a function of environmental parameters for comparison with the measurements. We would also like to proceed with the studies on the metastable solid-liquid equilibrium of water by improving the packaging of the sensor and the experiment set-up, or by modifying the design of the current generation of µTM to measure tension under lower temperature (i.e., -10°C or lower).
Silicon Nitride Cantilevers for Muscle Myofibril Force Measurements

CNF Project Number: 1255-04
Principal Investigator: Walter Herzog
Users: Timothy Leonard, Andrew Sawatsky

Affiliation: Faculty of Kinesiology, University of Calgary, Calgary, Canada
Primary Sources of Research Funding: Natural Sciences and Engineering Research Council of Canada, Canadian Institutes of Health Research and the Canada Research Chair for Cellular and Molecular Biomechanics
Contact: wherzog@ucalgary.ca, leonard@ucalgary.ca, ajsawats@ucalgary.ca
Website: www.ucalgary.ca/knes
Primary CNF Tools Used: GCA 5x Stepper, Oxford 81, Si,N, furnace, critical point dryer system

Abstract:
To measure muscle forces in the nano-Newton range, silicon nitride cantilever pairs were manufactured using the GCA 5x-Stepper photolithography system and the Oxford 81 ion etching system at the CNF, and then used in our lab in Canada. We investigated titin mechanical properties using a skeletal muscle myofibril model. Our experiments demonstrate that in myofibrils at long sarcomere length, the free-spring length of titin, which is present in the I-band region of the sarcomere, is an adjustable spring and allows for a tunable length.

Summary of Research:
Titin is a giant molecular spring present within the sarcomere and is responsible for most of the passive force found in muscle. Titin has elements with different stiffness and visco-elastic properties that come into play in an orderly fashion with increasing sarcomere length. In skeletal muscle, the I-band region of titin contains two distinct immunoglobulin (Ig) domains, a small N2A portion, and the PEVK segment [1]. Lengthening a skeletal muscle sarcomere within the physiologically relevant range first causes the Ig domains to straighten out and is then followed by extension of titin's PEVK domain. Both of the Ig regions (proximal and distal) and the PEVK region are thought to be essentially elastic at physiologically relevant sarcomere lengths (SL). However, at SL greater than the physiological range, Ig domains start to unfold during stretch, and this unfolding is thought to be responsible for titin then behaving in a highly visco-elastic manner.

Previous work by our group has shown that elevated passive forces in activated and lengthened muscle at very long SL (when myofilament overlap is lost) are much higher than the purely passive forces at matched SL [2]. A proposed mechanism for this is that the free-spring length of the titin is shortened by titin-actin interactions, thereby effectively producing a shorter and hence stiffer spring. If this is the case, then the question arises, does this interaction occur at a specific location on both filaments (titin and actin)? If so, then the free-spring length of titin has only two configurations; one long with no interactions (as in the passive state), and one shorter, due to interactions (in the active state). A 2-state model would mean that at long SL in active and then lengthened muscle, all forces should be similar since all free-spring lengths are similar.

Myofibrils were harvested from psoas muscle obtained from New Zealand White rabbits and were chemically and mechanically isolated as described in our previous work [3]. Single myofibrils were attached to nanofabricated silicon-nitride cantilevers (stiffness 68pN/nm) [4] for force measurement at one end of the myofibril (resolution < 0.5 nN), and at the other end, a glass pipette needle attached to a piezo-motor for controlling specimen length (Figure 1). Testing was done using an activating solution that contained ATP and free calcium with a pCa of 3.5. Passive data was provided by previous experiments [2]. Submaximal active forces were obtained by adding BDM (2,3-butanedione monoxime) to the activating solution. Myofibrils were either fully activated (n=1) or submaximally activated (n=4), and then stretched from an average sarcomere length of 2.6 µm to a final SL of approximately 5 µm at a speed of 0.1 µm/s/sarcomere. At a long SL in this case 4.5 µm, stress was measured for all samples so as to provide a matched SL for all tests. Stress was reported by normalizing force with the cross-sectional area of each myofibril.
Figure 2 (upper panel) is the stress-time data for two of the tests. Note that in the fully activated (control) test, the stress is substantially higher compared to one of the activated (with BDM) trials. Figure 2 (lower panel) shows all five activated trials and at matched sarcomere lengths, (4.5µm), the stress values range from just above the passive values (approximately 175nN/µm²) up to fully activated at 425 nN/µm². Myofilament overlap in psoas myofibrils is lost at SL beyond 4 µm, and so forces at these long lengths are not the result of cross-bridge cycling, and can only be attributed to titin.

From our results, we conclude that the I-band free-spring length of titin appears to be variable. The variation in stress for specimens at the same length and at very long SL (where active cross-bridge cycling is not possible) can only be explained by a variation in the titin free-spring length. The idea that the titin-actin attachment is a 2-state model only must be incorrect and so the sites for the interaction between these proteins must occur along the length of one or maybe both. If this is so, then the tunability of the free-spring length of titin poses interesting questions for future research.

References:
Biomimetic Models of the Tumor Microenvironment and Angiogenesis

CNF Project Number: 1540-07, 1278-04
Principal Investigators: Claudia Fischbach-Teschl, Abraham D. Stroock
Users: Peter DelNero, Lu Ling, John Morgan

Abstract:
Cancer is a disease of nature and nurture. On the one hand, oncogenic mutations drive the hyperproliferation of malignant cells. On the other hand, tumor growth and metastasis is intimately dependent on the surrounding tissue microenvironment that the cancer cells inhabit. Microvascular perfusion is a critical determinant of this environment. The microcirculatory system mediates the exchange of respiratory gases, metabolites, and drugs. In cancer, abnormal blood flow results in a highly heterogeneous tissue environment, which drives the evolution of increasingly adaptive cancer cell phenotypes. Our project uses tissue engineering and microfluidic approaches to unravel the complex interdependence between cancer cells and the vascular microenvironment, specifically in the context of tissue transport and tumor metabolism.

Summary of Research:
As the primary conduit of oxygen, nutrients, drugs, and other biomolecular signals, the vasculature is a critical determinant of the tumor microenvironment. To explore the interactions between cancer cells and the peripheral blood vessels, we integrated cancer biology and tissue engineering strategies to fabricate artificial tumor mimetics with explicit microvasculature, fully embedded within remodelable hydrogel scaffolds. We are using these in vitro models to investigate: 1) the regulation of vascular function and angiogenesis by cancer cells; 2) how microvascular dysfunction disrupts homeostatic tissue perfusion; 3) how populations of tumor cells adapt their metabolic phenotype in response to aberrant transport gradients [1]. By studying tissue-level phenomena, we hope to identify novel insights on disease progression that may inform a more effective use of existing anti-cancer treatments.

To investigate these questions, we use biomaterials-based microfluidic platforms to recapitulate the tumor-vascular microenvironment. In our previous report, we demonstrated the fabrication of endothelialized microvascular structures that displayed membrane barrier function and angiogenic sprouting. This year, we are integrating localized tumor compartments to create a more sophisticated co-culture system. This model allows precise control of microenvironmental...
conditions and *in situ* analysis of cell status. Moving forward, we aim to use the co-culture model to correlate the emergence of heterogeneous phenotypes within the asymmetric microvascular landscape. If successful, this approach will provide a tool to address previously inaccessible questions about tumor adaptation and evolution during disease initiation, progression, treatment, and recurrence.

In addition, we used soft-lithography to generate a collagen-based microwell platform to study the role of extracellular matrix remodeling in tumor angiogenesis [2,3]. Specifically, adipose stromal cells (ASCs), a major stromal component of mammary microenvironment, simultaneously deposit and contract the tumor matrix. In addition, these cells generate potent pro-angiogenic factors that promote blood vessel growth. We have previously shown that in tumor, ASCs become myofibroblastic cells that exhibit increased matrix remodeling and VEGF secretion. To evaluate the relative contributions of matrix structure and soluble factor secretion, we utilized microfabricated biomaterial-based assays to assess 3D endothelial cell sprouting. Our findings indicate that matrix remodeling by ASCs can be as equally, or even more important than, pro-angiogenic factor secretion in facilitating new vessel growth.

**References:**


Stamping and Twisting: Using Bio-Nanotechnology to Enhance Single Molecule Experiments

CNF Project Number: 1738-08
Principal Investigator: Michelle D. Wang¹
Users: Ryan Badman¹, James Baker¹,², Xiang Gao¹,²

Affiliations: 1. Department of Physics, Cornell University, Ithaca NY; 2. Howard Hughes Medical Institute, Chevy Chase, Maryland

Primary Source of Research Funding: Howard Hughes Medical Institute
Contact: mdw17@cornell.edu, rpb226@cornell.edu, jeb94@cornell.edu, xg237@cornell.edu
Website: http://wanglab.lassp.cornell.edu/

Primary CNF Tools Used: ASML Deep UV stepper, Oxford 100, PT 72, Trion chrome etcher, MVD 100 molecular vapor deposition, Zeiss Supra SEM

Abstract:

Traditional, single laser optical tweezer deoxyribonucleic acid (DNA) experiments trap, within the focal region of an infrared laser beam, a floating micro- or nanoparticle that is tethered to a glass surface by a single DNA molecule. A DNA tether can be stretched by pulling on the trapped particle, and resulting force-extension spectra can be analyzed to characterize the fundamental properties of bare DNA, or protein-DNA complexes.

The Wang lab is exploring more advanced capabilities beyond the traditional approach discussed above, through two bio-nanotechnology methods: (1) using nanostamps to precisely pattern protein anchor points on a microscope slide for DNA to tether in organized arrays, and (2) using birefringent quartz nanocylinders in angular optical traps to add “twisting” capabilities to optical tweezers that previously could only stretch. The twisting capability of angular optical traps allows measurement of “torque spectra” that provide additional fundamental biophysical information beyond the traditional force-extension spectra generated in DNA stretching.

Summary of Research:

With the goal of pursuing high impact, fundamental biology experiments, the Wang lab has leveraged nanofabrication technologies to augment traditional optical tweezer experiments. We have been pushing beyond the traditional approaches through two nanotechnology methods: (1) using nanostamps [1] to precisely pattern protein anchor points on a glass surface so that DNA molecules are tethered with greater control, and (2) trapping birefringent quartz nanocylinders in angular optical tweezers [2] to add “twisting” capabilities to optical tweezers that previously could only stretch. The twisting functionality allows analysis of “torque spectra” that provide additional fundamental biophysical information beyond the traditional “force-extension spectra” generated in DNA stretching experiments.

For the nanostamping method, polydimethylsiloxane (PDMS) nanostamps (Figure 1) give the ability to study the interactions between two DNA tethers by precisely controlling the distance between the stamped protein anchor positions. The protein anchors will be spaced...
with the same array pitch as the tips of the pyramid on the PDMS stamp. The nanostamps can also increase the local anchor protein density without increasing the overall tether density, which is useful for experiments involving torsionally constrained tethers. The nanostamps can enhance sample preparation outcomes for traditional optical tweezer experiments, angular optical trap studies, and also be used in conjunction with magnetic tweezer setups [3].

For the birefringent trapping targets, while the Wang lab has previously published results using birefringent quartz cylinders [2] for DNA twisting, recently we have been developing newer, faster and highly reproducible protocols using the ASML Deep Ultraviolet (DUV) stepper and the Oxford 100 in CNF to fabricate quartz cylinders with a higher quality than was achieved a decade ago using less advanced nanofabrication tools. Figure 2 shows an example image of recently made quartz cylinders. See Figure 3 for a schematic depicting an example of our experimental setup: a trapped quartz cylinder tethered with DNA to a glass surface.

References:
Biocompatible and High Stiffness Tunable Nanophotonic Array Traps with Enhanced Force and Stability

CNF Project Number: 1738-08
Principal Investigator: Michelle D. Wang
Users: Fan Ye, Ryan Badman

Affiliations: 1. Department of Physics, Cornell University; 2. Howard Hughes Medical Institute, Chevy Chase MD
Primary Source of Research Funding: Howard Hughes Medical Institute
Contact: mdw17@cornell.edu, fy72@cornell.edu, rpb226@cornell.edu
Website: http://wanglab.lassp.cornell.edu/

Primary CNF Tools Used: ASML deep UV stepper, Oxford 100, Unaxis 770, Heidelberg DWL2000, SÜSS MA6-BA6, Gamma automatic coat-develop, LPCVD Nitride - B4, Wet/Dry Oxide - B2, AJA sputter deposition, GSI PECVD, Oxford PECVD, SC4500 odd-hour evaporator, Zeiss Supra SEM, Zeiss Ultra SEM

Abstract:
A nanophotonic trapping platform based on on-chip tunable optical interference allows parallel processing of biomolecules and holds promise to make single molecule manipulation and precision measurements more easily and broadly available. The Wang lab has developed and implemented such an on-chip device based on silicon (Si) or silicon nitride (Si$_3$N$_4$) waveguides, coined a nanophotonic standing-wave array trap (nSWAT), that allows for controlled and precise manipulation of trapped nano/micro particle arrays [1,2]. By taking a systematic design approach, we present here a new generation of nSWAT devices with significant enhancement of the optical trapping force, stiffness, and stability, while the quality of the standing wave trap is resistant to fabrication imperfections [3]. The device is implemented on a Si$_3$N$_4$ photonic platform and operates at 1064 nm wavelength that permits low optical absorption by the aqueous solution. Such performance improvements open a broader range of applications based on these on-chip optical traps.

Summary of Research:
Optical trapping is a powerful manipulation and measurement technique widely employed in the biological and materials sciences. Miniaturizing bulky and expensive optical trapping instruments onto optofluidic platforms holds promise for high throughput lab-on-chip applications that can be readily integrated with other novel lab-on-chip innovations such as fluorescent detectors or on-chip lasers.

Recently, we have demonstrated a high-throughput, near-field nanophotonic trapping platform that achieved stable trapping with precision controllable repositioning [1-3]. The core concept of the platform is nanophotonic standing-wave interferometry, where laser light travels through a nanophotonic waveguide, is split into two equal intensity laser beams, the two beams are guided by the waveguides and meet each other, which ultimately leads to interference of two counter-propagating laser beams and results in the formation of standing waves. The evanescent field of the antinodes of the standing wave forms an array of stable three-dimensional optical traps. We call this type of trap a nanophotonic standing-wave array trap (nSWAT). By

Figure 1: Taken from [3]. nSWAT analysis and fabrication. a) A schematic of the device layout. The electric field vectors at different locations along the waveguide are indicated. b) An optical microscope image of the fabricated device.
tuning the phase difference between the two counter-propagating laser beams, the antinode locations can be precisely repositioned, and consequently, the optical trap positions can be precisely manipulated. The nSWAT device holds the capability for high throughput precision measurements on-chip.

In the past year, we developed a new nSWAT design that significantly improves the stability of the trap and doubles the trapping force and energy for the same input laser power. For a quantitative comparison, we fabricated both the old and the new trap design on the same chip and compared their performance concurrently under the same input laser power. We demonstrated that this new force-doubling design is more resistant to fabrication imperfections than the old design. The CMOS compatible fabrication procedures and numerical simulation details of the nSWAT devices are documented in Refs. [2,3].

Figure 1 (taken from [3]) depicts the structure of the trapping device that contains both the old nSWAT and the new nSWAT design in proximity of each other and operate by the same laser power. Figure 2 (taken from [3]) shows the scattered laser light from the waveguide at both locations to demonstrate the force-doubling nature of the new design. Figure 3 (taken from [2]) shows the stiffness measurement of an array of polystyrene beads trapped on the Si$_3$N$_4$ waveguide to demonstrate the built-in capacity for parallelized precision manipulation and measurements of an nSWAT device.

Our development and improvement of the Si$_3$N$_4$ waveguide nSWAT platform has led to three publications in the past year, see Refs. [2-4].

References:
Generalized Microfluidic Immunosensor for Antibody Detection

CNF Project Number: 1757-09
Principal Investigator: Christopher K. Ober
Users: Aibar Nurmukhanov, Roselynn Cordero

Affiliations: 1. Department of Materials Science and Engineering, 2. Department of Chemistry and Chemical Biology; Cornell University
Primary Source of Research Funding: National Science Foundation
Contact: cko3@cornell.edu, an485@cornell.edu, rc634@cornell.edu
Primary CNF Tools Used: ABM contact aligner, Hamatech-Steag Wafer Processor, CVC SC4500, DISCO dicing saw, CorSolutions fluidic probe station

Abstract:
Microfluidics are important devices that control and manipulate fluid flows with volume sizes ranging from microliters to picoliters. These devices received enormous attention in the fields of biology and biotechnology for the recent development of sensing devices that manipulate, analyze, and detect small quantities and operate at smaller volumes. Microfluidics offer numerous advantages such as ability to work with small quantities, potential for cheap and portable immnosensor device fabrication, manipulation of multiple samples at once, and applicability for patient treatments in isolated areas with no laboratory settings, facilities, and well-trained technicians. Microfluidics can also be used as point-of-care systems for diagnostics that provide real clinical value, and help identify and treat serious illnesses such as diabetes or cancer. These lab-on-a-chip (LOC) devices demonstrate reliable diagnostic results and practical application to replace some laboratory tests. This report focuses on the master mold fabrication using different SU-8 permanent epoxy negative photoresist using photolithography and replication of microchannels using polydimethylsiloxane (PDMS) block for LOC applications.

Summary of Research:
A patterned device, consisting of 35 rows of seventeen fused silica squares (300 x 300 µm) surrounded by a patterned gold lines (150 µm wide) was fabricated using lift-off process (Figure 1). A lift-off process includes spin-coating a bilayer of photoresists: lift-off resist LOR 10A (based on polydimethylglutarimide) and positive-tone SPR220-3.0 photoresist (based on cresol novolak resin and diazo photoactive compound) on fused silica substrate followed by baking at 180°C for 3 minutes and 115°C for 1.5 minutes, respectively. An ABM contact aligner with mercury arc lamp (i-line: 365 nm optical source) was used to expose a positive photoresist for 10 seconds followed by post-exposure bake at 115°C for 1.5 minutes. Then, the substrate was developed by Hamatech-Steag Wafer Processor using a double puddle automated process in tetramethylammonium hydroxide (TMAH) solution to obtain an undercut profile for metal deposition. Subsequently, 15 nm of chromium (adhesion layer) and 100 nm of gold layers were deposited on the wafer using a CVC SC4500 electron beam evaporation system.

Figure 1: Generalized platform made of fused silica substrate and generated pattern of thin layer of gold lines by lift-off process.
A solution of Remover 1165 was used to remove photoresist and obtain isolated patterns of gold lines as shown in Figure 2. The whole wafer was cut into six individual platforms using an all-purpose blade on DISCO-dicing saw. The same positive photoresist (SPR220-3.0) was spin-coated on the wafer as a sacrificial layer before dicing.

Microfluidic channels were fabricated using epoxy-based SU-8 type negative photoresist by master mold fabrication using photolithography (Figure 2). Preparation of the PDMS and replication of master mold patterns on its surface is done via widely known and relatively simple soft lithography process. PDMS was prepared using polymer base and curing agent, then poured over SU-8 master mold structure and cured in the oven at 60°C for at least two hours. Finally, a PDMS replica with microchannels was peeled away from the substrate to get embossed microstructure.

Polydimethylsiloxane (PDMS) was chosen due to its chemical and physical properties that suit our immunosensor design. PDMS is a non-toxic, cheap and easy-to-mold elastomer making it a strong candidate for integration in portable and inexpensive immunosensor device. Sealing of the PDMS to the surface of the platform was achieved by evaporation of the ethanol between the surfaces of the platform and PDMS replica (Figure 3). This method is non-destructive compared to other conventional methods such as oxygen plasma or corona discharge. Methods that use harsh conditions of oxygen plasma tend to make strong (covalent) bonding between the glass and the PDMS. However, these methods also change and sometimes destroy crucial surface chemistry of the platform, which is needed for successful implementation of the fundamental idea of the antibody catalyzed water oxidation pathway process.

**References:**


A Portable, Quantitative Nucleic Acid Amplification System for Disease Diagnostics in Limited Resource Settings

CNF Project Number: 1858-10
Principal Investigator: David Erickson
User: Ryan Snodgrass

Affiliation: Mechanical Engineering, Cornell University
Primary Source of Research Funding: National Institutes of Health
Contact: de54@cornell.edu, rjs492@cornell.edu
Website: ericksonlab.org
Primary CNF Tools Used: VersaLaser laser cutter

Abstract:
We built a portable diagnostic device for limited resource settings called TINY. TINY performs isothermal nucleic acid amplification with the flexibility of being heated via non-electricity sources if electricity is unavailable (e.g., sunlight or open flame). TINY is also capable of nucleic acid quantification, therefore delivering a technology that is usually contained in a large, immobile laboratory tool to a handheld package weighing just over 1 kg.

Summary of Research:
Nucleic acid tests (NAT) are still largely inaccessible to rural communities in resource limited settings [1]. While PCR (polymerase chain reaction) and qPCR (quantitative PCR) are common diagnostic techniques in expensive laboratory settings, delivering the same diagnostic capability to developing countries calls for unique engineering solutions. Our research during the past year has culminated in the production of TINY, a device for performing NAT in the field using alternative heat sources such as sunlight and open flame. Our lab previously developed a microfluidic chip capable of performing PCR using sunlight, but it was incapable of DNA quantification [2,3]. Other groups have developed portable and quantitative NAT using an isothermal variant of PCR known as LAMP (loop-mediated isothermal amplification) [4], but these devices are designed to be heated via exothermic chemical reaction, and cannot use electricity when available [5].

TINY (Tiny Isothermal Nucleic acid amplification sYstem) is a portable tool capable of performing quantitative, isothermal nucleic acid amplification. TINY is unique in that it may be heated via solar energy (Figure 1), flame (from a Bunsen burner), or electricity. This makes it particularly suited for use in locations where electricity is only occasionally accessible or completely inaccessible. TINY tracks fluorescence and absorbance of four samples simultaneously, is designed to be used with standard PCR tubes as sample containers, weighs only 1.1 kg, and occupies just over 2 L of space. It is easily carried in one hand. The optical design in TINY is unique in that only one, stationary excitation source is used and no mechanical actuation is required to track the optical characteristics of the multiple samples simultaneously.

A large concern when using sunlight as a heat source for diagnostic tools is the irregular solar irradiance and its effect on sample temperature. Our previous microfluidic...
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Biological Applications

Device [2,3] showed large temperature fluctuations due to even small changes in weather (e.g., cloud coverage). We therefore designed TINY to store large amounts of heat isothermally in the latent heat of a phase change material. We store approximately 14 kJ of latent heat in 75 mL of a phase change material that melts at 68°C, a temperature optimal for LAMP. If enough heat is stored, the device can maintain isothermal conditions for about an hour even while no longer collecting any heat (Figure 2).

We are currently performing a large trial on more than 40 biopsy samples collected in Kampala, Uganda, from patients suspected of having Kaposi's sarcoma (KS) — a cancer most common in HIV-positive individuals in sub-Saharan Africa. Our results show that TINY can reliably track the fluorescence or absorbance (Figure 3) of multiple samples in real-time allowing for the quantification of target DNA. We have also discovered that TINY has quantitative capability on-par with commercial nucleic acid amplification systems that can perform LAMP, meaning that TINY is capable of performing tests for other nucleic acid targets and that the performance of the diagnostic is limited by the specific assay in use and not the TINY itself.

TINY was designed and built on Cornell’s campus. We used the Cornell NanoScale Science and Technology Facility for assistance with fabrication of many of the parts that are used in the construction of TINY. For example, the Versa laser cutter was essential in cutting opaque parts that are necessary for the optical function in the system.

References:
Fabrication of Elastomeric Microposts with Step-Changes in Rigidity

CNF Project Number: 1859-10
Principal Investigator: Cynthia A. Reinhart-King\(^b\)
User name: Jacob VanderBurgh\(^a,b\)

Affiliations: a. Meinig School of Biomedical Engineering, Cornell University, Ithaca, New York, USA; b. Department of Biomedical Engineering, Vanderbilt University, Nashville, Tennessee, USA

Primary Source of Research Funding: National Science Foundation Award 1435755
Contact: cynthia.reinhart-king@vanderbilt.edu, jav244@cornell.edu
Website: http://www.cellmechanics.org/
Primary CNF Tools Used: AutoStep GCA i-line stepper, Unaxis 770 Deep Si etcher, Heidelberg DWL 2000, ABM contact aligner

Abstract:
Arterial stiffening occurs during aging and rigidity sensing of the vascular endothelium plays an important role in atherosclerosis. Recent evidence indicates arterial stiffening is accompanied by greater heterogeneity in stiffness within the intima. To explore the effect of heterogeneity in substrate stiffness upon the endothelium, we fabricated dense arrays of elastomeric micropillars that introduce multiple subcellular step changes in substrate rigidity. Endothelial cell area increased with increasing rigidity, verifying cells respond appropriately to the range of rigidities provided. Future work will focus on further characterizing endothelial barrier integrity in response to spatial changes in rigidity.

Summary:
Cardiovascular diseases are the leading cause of death worldwide and cardiovascular risk can be predicted by vascular stiffness [1,2]. Vascular stiffening occurs with age and is correlated with traditional cardiovascular risk factors such as hypertension [3,4]. Our lab and others have shown that vascular stiffening regulates vascular endothelial permeability permitting cholesterol uptake and leukocyte extravasation into the vessel wall, hallmarks of atherosclerosis [5-7]. Recent evidence indicates that vascular stiffening is accompanied by greater heterogeneity in the rigidity of the intima with areas of high stiffness located directly adjacent to areas of low stiffness [8].

To explore the consequences of heterogeneous intimal stiffening, we have developed a micropillar model to explore the impact of greater complexity in substrate stiffness upon endothelial cell function.

Negative silicon (Si) masters of micropillars with a step-change in rigidity were fabricated with two-stages of deep reactive-ion etching (DRIE) as described previously (Figure 1) [9]. RCA-cleaned Si wafers with a thin layer of photoresist were first patterned with a 5x reduction stepper and a photomask consisting of a hexagonal array of holes. Patterned photoresist was used as a mask for subsequent DRIE on a Unaxis 770 Deep Si Etcher to generate an array of hexagonal holes of uniform depth. Hole depth was controlled by monitoring etch time and utilized to fabricate a library of silicon masters with varying uniform hole depth.

Thick photoresist was subsequently spun onto these arrays to completely fill the cylindrical holes and rectangular strips of photoresist were patterned with an ABM contact aligner. Photoresist was developed such that photoresist dissolved from the surface of the Si wafer, but remained within the cylindrical holes,
protecting the bottom of the cylindrical holes during the second DRIE step. Replica molding of the Si negative master with silicone elastomer polydimethylsiloxane (PDMS) yields coplanar micropillars with regions of shorter (stiff) and taller (compliant) pillars (Figure 2). Pillar height corresponds to perceived substrate stiffness \( E_{\text{eff}} \) as described in Equation 1 in which \( E \) is the bulk PDMS modulus of elasticity, \( r \) is the pillar radius, and \( L \) is the pillar height [10]. Micropillar substrates were coated with fibronectin and seeded with bovine aortic endothelial cells (BAEC).

Projected cell area of subconfluent BAECs increased with increasing rigidity, which is consistent with previously reported findings and validates endothelial cells are responding appropriately to the range of micropillar rigidities examined (data not shown) [11]. Future work will characterize endothelial barrier integrity in response to complex cues in substrate rigidity.

**References:**


Abstract:

Point of care diagnostic devices allow people to get fast, accurate information about their health and well-being without the need to go to a clinic or hospital. The device that we are designing will determine the concentration of cortisol from a sample of the user’s saliva. Cortisol is a steroid hormone associated with stress levels and expressed in human saliva [1,2]. This microfluidic device will contain a microbead-based immunoassay that will determine the cortisol content from a saliva sample. The device is manufactured using a hot embossing process, which uses a silicon master made with traditional lithographic processes. The device will be made from a thermoplastic called Zeonor 1020R, which is a transparent, semi-rigid plastic that can be used in large-scale manufacturing processes such as injection molding and hot embossing. All of the fabrication of the device is being done in the Cornell NanoScale Facility.

Summary of Research:

The microfluidic device is made using a hot embossing process, which involves the high-temperature pressing of a mold into a piece of thermoplastic. The mold used in this process is made of silicon and is fabricated using photolithographic processes. The design for the mold is made using L-Edit and transferred to a photomask using the Heidelberg Mask Writer (DWL2000). This mask is then used to transfer a pattern to a photoresist on a silicon wafer. The photoresist (SPR-220-7.0) is spun onto a bare silicon wafer, which has been previously primed in the YES Vapor Prime Oven, to a thickness of approximately 7 µm. After spinning, the photoresist is soft baked on a 115°C hot plate for 2 minutes and 30 seconds. The wafer is allowed to sit for an hour and then exposed using the mask and the ABM Contact Aligner. The wafer is again allowed to sit for an hour and then is developed using the Hamatech Steag Wafer Processor. The pattern is now developed and can be used to etch the silicon wafer.

We etched the wafer using the Unaxis 770 Deep Si Etcher to a depth of 100 µm, and monitored the etch depth and etch rate using the P10 Profilometer. Upon reaching the desired depth, we removed the photoresist in the chemical strip bath. In order to ensure that the process was working as desired, we used the scanning electron microscope (SEM) to image the various features of the device. An SEM image of the smallest features, around 20-40 µm in size, can be seen in Figure 1. We then used the Unaxis 770 again to deposit a thin layer of fluoropolymer onto the wafer in order to prevent sticking in the hot emboss process. In order to separate the individual patterns into separate masters, we cut the wafer into pieces using the DISCO Dicing Saw. Masters are then ready to be used in the hot emboss process. Some of these masters can be seen in Figure 2.
The hot emboss process uses the CRC Prepreg Mini Test Press, which applies heat and even pressure. The silicon master is adhered to a glass backing, for strength, and then the plastic piece is placed on top of the master, with another glass backing on top of that. This whole stack is placed in the hot press once the hot press reaches the desired temperature and pressed for several minutes. The setup is allowed to cool below the glass transition temperature of the plastic and then the pressure is released and the plastic is de-embossed. The pattern is transferred from the master to the plastic. We then drill through-holes in a blank piece of plastic using the custom-made micro drill. This blank piece is then bonded to the patterned piece to create the microfluidic device. For the bonding process, we used the Harrick Plasma Generator to activate the surfaces of the plastic pieces and immediately put the pieces together and put them in the hot press again to thermally bond.

The microfluidic device is now complete and ready to be turned into an immunoassay. A completed device with blue dye shown for visualization of the channels can be seen in Figure 3.

References:
**Increasing Endothelial Retention on Mechanical Heart Valves via Surface Micropatterning**

**CNF Project Number:** 1923-10  
**Principal Investigator:** Dr. Jonathan Butcher  
**User:** Brett Resnick

**Affiliation:** Biomedical Engineering Department, Cornell University  
**Primary Source of Research Funding:** Cornell University  
**Contact:** JTB47@cornell.edu, BER62@cornell.edu  
**Primary CNF Tools Used:** CVC SC4500, PlasmaTherm deep Si etcher, Zygo optical profilometer, DISCO dicing saw, Heidelberg DWL2000

**Abstract:**
In treating heart valve disease, physicians try to avoid using mechanical heart valves because of the lifelong anticoagulation therapy they require. Endothelial cells (EC) have antithrombotic properties, which studies suggest can be used to reduce clotting on valve surfaces if they can tolerate severe flow conditions. Success with EC retention was found by creating devices with microtrenches to reduce surface shear. Using the Cornell NanoScale Facility, we aimed to fabricate constructs with microchannels across the surface that would sufficiently reduce EC detachment during flow. After fabrication, endothelial adhesion to these constructs would be tested using shear flow bioreactor experimentation. To create these artificial valves, we patterned resist on a 1 mm thick Si wafer and deposited alumina. The resist was removed such that only an alumina etching mask remained, after which 700 µm trenches were etched into the wafer. Then pyrolytic carbon was deposited and, finally, the trenched wafer was cut into 1 cm² square pieces. Resulting images suggest that our micropatterned devices successfully reduce the shear stress experienced by the EC, thus improving their adhesion to the valve surface.

**Summary of Research:**
The role of heart valves is to ensure the unidirectional flow of blood through the body during circulation [1]. Therefore, any mineralization, degeneration, or damage to heart valves can lead to excessive regurgitation, insufficient circulation, and overall heart failure. Currently, the traditional method of treatment, and last line of defense, for valvular heart disease involves implanting one of two replacement valve types: mechanical or biological [2]. While mechanical heart valves have the superior durability and strength for a longer implantation lifespan, the biomaterials from which they are composed often struggle with hemocompatibility [2].

Endothelial cells are known for their ability to counteract this issue with hemocompatibility, and can therefore be used as a successful antithrombotic coating on mechanical heart valves. However, endothelial adhesion under cardiovascular flow conditions remains an issue. Frendl, et al., showed how patterning a surface with micro-trenches can reduce the shear effect of fluid flow on endothelial cells, and therefore increase their ability to adhere to the surface [2]. This study aimed to determine a standard fabrication process for these microchanneled constructs. In coordination with the Cornell NanoScale Facility (CNF) and its staff, we micropatterned the surface of a silicon wafer, in order to produce constructs similar to those created by Dr. Mandy Esch in the Frendl study. Unfortunately, due to technological changes and instability of previously made masks, a new fabrication process had to developed.

In attempting to use the older masks, all surface features began to erode once the etching process reached a depth of approximately 450 µm. Additionally, there was great variation in channel depth throughout the wafer, resulting in major dimensional inconsistencies between samples. An alternate attempt at device fabrication involved the wet etching of an alumina mask. However, this process led to significant lateral undercutting of the mask, in addition to deformation of the microchannels, themselves.
Eventually, the CNF team was able to agree upon a potential new method. The fabrication procedure would begin with a 1 mm thick SiO2 wafer, 100 mm in diameter.

First, the oxide was removed from the initial wafer using hydrofluoric acid. Once oxide-free, the wafer was layered with a negative photoresist (nLOF 2020), whose pattern would serve as a guide for alumina deposition in the proceeding step. Once the resist was properly situated, a coating of alumina (aluminum oxide, Al2O3) was deposited on the wafer using the CVC SC4500 e-gun evaporation system for thin films. The treated wafer was then soaked for two days in lift-off solution, that would remove the photoresist layer, thus leaving only the desired alumina etching mask behind (Figure 1).

Once lift-off of the negative resist was complete, the wafer was subjected to DRIE silicon etching within the PlasmaTherm Deep Si Etcher. Channel depths were measured after each round of cycling using a Zygo optical profilometer (Figure 2). As the trenches reached a depth of 700 µm, the wafer was once again placed in the CVC SC4500 to deposit a pyrolytic carbon coating of approximately 75 nm. Finally, following the patterning created using the alumina etching mask, the wafer was cut into 1 cm² square pieces using a DISCO dicing saw.

This entire process was conducted within the class 1000 particle-controlled environment of the CNF cleanroom. The final product can be seen in Figure 3.

References:
Microconcentrator for Biological Small Angle X-Ray Scattering

CNF Project Number: 1940-10
Principal Investigator: Richard E. Gillilan
Users: Manjie Huang, Melanie MacMullan

Affiliations: Macromolecular Diffraction Facility of the Cornell High Energy Synchrotron Source (MacCHESS), Cornell High Energy Synchrotron Source; Cornell University
Primary Source of Research Funding: National Institutes of Health GM-103485
Contact: reg8@cornell.edu, mh2334@cornell.edu, mm2354@cornell.edu
Website: www.macchess.cornell.edu/MacCHESS/bio_saxs.html
Primary CNF Tools Used: VersaLaser laser cutter

Abstract:

Biological small angle x-ray solution scattering (BioSAXS) continues to enjoy widespread popularity as a means of characterizing the structure and behavior of biomolecules in solution. BioSAXS requires not only a minimum concentration to obtain statistically useful scattering signals, but a full range of concentrations to characterize the solution behavior. Many important systems examined by today’s researchers are difficult to prepare in quantity and are only marginally soluble. Attempts to concentrate such samples often result in aggregation and subsequent sample loss. In situ concentration of samples at the synchrotron beamline allows researchers to explore the full range of concentrations with minimal sample loss. Semipermeable membranes embedded into a modified microfluidic BioSAXS sample cell provide a means of concentrating protein solutions in close proximity to the x-ray beam. Preliminary studies have demonstrated that this concentration process can be achieved on practical time scales at modest pressures without damage to sensitive thin x-ray windows or the formation of leaks.

Summary of Research:

Solubility of complex biological preparations can often be problematic. High concentrations shift equilibrium in favor of the formation of higher oligomers or aggregates. When samples are precious, the point at which sample aggregation occurs is usually not known a priori. On the other hand, low concentrations can favor the dissociation of important complexes and contribute to poor x-ray scattering signal. In earlier work [1], we explored concentrating samples at ambient pressure using dialysis. Dialysis and ultrafiltration are closely related techniques. The latter method relies upon pressure to drive separation of biomolecules from solvent rather than diffusion alone. The use of pressure, however, is problematic in x-ray scattering due to the necessity of having very thin x-ray transparent windows. To explore the feasibility of in situ ultrafiltration during x-ray solution scattering, students Manjie Huang and Melanie MacMullan adapted our currently-used in-vacuum BioSAXS flow-cell design to incorporate a semipermeable (ultrafiltration) membrane.

Figure 1: Ultrafiltration chip design and flow scheme. Stack of PMMA sheets allows sample delivery on one side of membrane and filtrate removal on the other (see inset A of B). A third outlet (large white arrow in B, leftmost tube in C) allows concentrated sample to flow past x-ray window.
Ultrafiltration membranes were sandwiched between multiple layers of PMMA using a design that incorporates standard chromatography (PEEK) tubing for inlets and outlets (Figure 1A-C). PMMA parts were fabricated on the VersaLaser system at CNF. Natural ruby mica (25 µm thick, Attwater, UK) was used for the x-ray windows to reduce distortion due to pressure. Monomeric bovine serum albumin (BSA) (SIGMA Life Science, A1900-500MG, lot SLM3178V) was prepared at 0.8 mg/ml for introduction into the chip, with real-time in-chip concentration levels determined by comparison of scattering intensity profiles to solutions of known concentration. Transmembrane pressures of 200-300 mbar produce concentration factors of 10-20 times over the course of less than an hour, a practical timescale similar to chromatography techniques in molecular biology.

After a period of concentration, samples are allowed to exit the membrane region of the chip to flow past the x-ray window. The total integrated x-ray intensity trace as the sample flows past the window is a single peak (Figure 3, grey line). Estimated molecular weight (Figure 3, grey dots) shows a dip at the peak, which is characteristic of high concentrations. COMSOL Multiphysics simulations of the concentration process (inset in Figure 3) show an expected concentration gradient near the x-ray window surface, a phenomenon that limits concentration uniformity in this design. Future designs will explore ways to uniformize concentration and minimize sample volume requirements. A timelapse video of the concentration process can be seen at http://news.chess.cornell.edu/articles/2016/Gillilan161213.html

References:
Microfluidic Mixer for Biological Small Angle X-Ray Scattering

CNF Project Number: 1940-10
Principal Investigator: Richard E. Gillilan
User: Jesse Hopkins

Affiliations: Macromolecular Diffraction Facility of the Cornell High Energy Synchrotron Source (MacCHESS), Cornell High Energy Synchrotron Source; Cornell University
Primary Source of Research Funding: National Institutes of Health GM-103485
Contact: reg8@cornell.edu, jbh246@cornell.edu
Website: www.macchess.cornell.edu/MacCHESS/bio_saxs.html
Primary CNF Tools Used: VersaLaser laser cutter, SUEX laminator, ABM contact aligner

Abstract:

Time resolved small angle x-ray solution scattering (TR-SAXS) has provided important new insights in structural biology, but the experiments have remained technically challenging. The so-called continuous-flow mixing strategy is beginning to emerge as the best way to make these types of experiments more practical and accessible to biologists visiting synchrotron beamlines. Based on the concept of chaotic advection mixing [1], using photolithographic methods at CNF, we have fabricated a continuous-flow microfluidic mixing chip that has allowed us to measure benchmark protein unfolding events below the 10 ms timescale.

Summary of Research:

Small-angle x-ray solution scattering (SAXS) is a structural probe for biological macromolecules capable of determining molecular weight, oligomeric state, conformational changes and flexibility under realistic physiological conditions. Time resolved SAXS (TR-SAXS) measures time dependent structural changes of macromolecules in solution and is often used to study conformational changes or unfolding/refolding. The most difficult part of time resolved SAXS is often initiating the reaction in the system. Typically, this is done by fluidic mixing that can change the pH, salt concentration, or other conditions.

Our mixing chip is based on the principle of chaotic advection [1]. As part of our initial design process, we used the COMSOL Multiphysics program to simulate flow and mixing (Figure 1A). We chose a mixer design covering a large range of flow rates (three orders of magnitude), allowing reactions with slower times to be probed with lower sample consumption. Actual fabrication was carried out at the Cornell NanoScale Science and Technology Facility (CNF). X-ray windows and liquid ports were cut in a PDMS backing. Polyimide (7.5 µm thick) was epoxied over the window hole and 500 µm thick SUEX was laminated over the acrylic. Patterning was done using photolithography. ADEX (5 µm) was used as an adhesive layer for a final polyimide layer to seal the mixing channel.

For testing, we measured the well-characterized urea-induced lysozyme refolding reaction [2]. The unfolding was initiated through a tenfold dilution of sample with 8M urea solution. Several time points were measured from 9ms to 1s immediately after mixing. Radius of gyration ($R_g$) is an easily-extracted parameter from SAXS data that measures the overall size of the protein. Figure 2 shows how our chip produces the characteristic decrease in $R_g$ with time that results from denaturation.

Based on this preliminary test, CNF-fabricated microfluidic mixing chips are expected to provide a basis for our future TR-SAXS user program at CHESS, helping to make time resolved biological scattering experiments practical for novice users with problems on a wide range of timescales.

References:

2016-2017 Research Accomplishments

Biological Applications

Figure 1: Microfluidic mixer for time-resolved biological small-angle solution scattering. Based on computational fluid dynamics simulations (A) we designed a chip capable of reaching a wide range of timescales relevant to structural biology. A prototype chip was constructed by photolithographic methods at CNF and tested at the G1 beamline of the Cornell High Energy Synchrotron Source (B).

Figure 2: First results from prototype microfluidic mixing chip. The radius of gyration \( R_g \) of the standard protein lysozyme changes in well-characterized ways when mixed with the denaturant urea. The experiment successfully captured protein unfolding behaviour on a timescale from 1s down to 9ms.
Design and Application of Microfluidic Devices to Study Cell Migration in Confined Environments

CNF Project Number: 2065-11
Principal Investigator: Jan Lammerding
User: Aaron Windsor

Affiliations: 1. Biomedical Engineering Department and Weill Institute, Cornell University; 2. Cornell NanoScale Science & Technology Facility (CNF)

Primary Sources of Research Funding: National Institutes of Health award R01 HL082792; National Institutes of Health award 1U54 CA210184; Department of Defense Breast Cancer Research Program Breakthrough Award BC150580; National Science Foundation CAREER award CBET-1254846

Contact: jan.lammerding@cornell.edu, ajw49@cornell.edu
Website: http://lammerding.wicmb.cornell.edu/

Primary CNF Tools Used: Heidelberg DWL2000, ABM contact aligner, YES polyimide bake oven, MVD100 molecular deposition, SU-8 hotplates

Abstract:

Metastatic spreading of cancer cells is responsible for the vast majority of cancer deaths. To better understand the biophysical processes and molecular consequences involved in cancer cell migration through tight spaces, we designed a novel microfluidic device capable of observing deformation of cells and their nuclei during confined three-dimensional (3-D) migration. Using these devices we observed substantial deformation of the nucleus, including strain on the chromatin, changes in the volume of the nucleus and loss of nuclear envelope integrity, which led to the uncontrolled exchanged of nucleo-cytoplasmic content, herniation of chromatin across the nuclear envelope and DNA damage. Our findings indicate that cell migration incurs substantial physical stress on the nuclear envelope and its content, and requires efficient nuclear envelope and DNA damage repair for cell survival.

Summary of Research:

The ability of cells to migrate through tissues and interstitial spaces is an essential factor during development and tissue homeostasis, immune cell mobility, and in various human diseases. Deformation of the nucleus and its associated lamina during 3-D migration is gathering increasing interest in the context of cancer metastasis, with the underlying hypothesis that a softer nucleus, resulting from reduced levels of lamin A/C, may aid tumour spreading. However, current methods to study the migration of cells in confining 3-D environments are limited by their imprecise control over the confinement, physiological relevance, and/or compatibility with high resolution imaging techniques.

We designed and built a polydimethylsiloxane (PDMS) microfluidic device composed of channels with precisely-defined constrictions mimicking physiological environments that enable high resolution imaging of live and fixed cells. The device promotes easy cell loading and rapid, yet long-lasting (>24 hours) chemotactic gradient formation without the need for continuous perfusion. Using this device, we obtained detailed, quantitative measurements of dynamic nuclear deformation as cells migrate through tight spaces,
revealing distinct phases of nuclear translocation through the constriction, buckling of the nuclear lamina, and severe intranuclear strain. Furthermore, we found that lamin A/C-deficient cells exhibited increased and more plastic nuclear deformations compared to wild-type cells but only minimal changes in nuclear volume, implying that low lamin A/C levels facilitate migration through constrictions by increasing nuclear deformability rather than compressibility.

We detected nuclear envelope rupture using previously established fluorescent reporters consisting of green or red fluorescent proteins fused to a nuclear localization sequence that rapidly escapes into the cytoplasm when nuclear envelope integrity is lost. Breast cancer, fibrosarcoma, and human skin fibroblast cells displayed transient loss of nuclear envelope integrity, which coincided with the nucleus passing through the constrictions. Nuclear envelope rupture was associated with transient influx of fluorescently labeled cytoplasmic proteins into the nucleus.

Irrespective of the experimental model, the incidence of nuclear envelope rupture increased exponentially with decreasing pore size and reached > 90% when the nuclear height was confined to 3 µm. Nuclear envelope rupture in vitro and in vivo was often accompanied by protrusion of chromatin through the nuclear lamina. The incidence of such “chromatin herniations” increased significantly with decreasing pore sizes. Furthermore, cells that had passed through microfluidic constrictions had more nuclear fragments positive for γ-H2AX, a marker of DNA double-strand breaks than cells that had not yet entered the constrictions. To assess the functional relevance of nuclear envelope repair, we quantified cell viability after rupture. Under normal conditions, the vast majority (>90%) of surviving cells experienced repeated NE rupture. Inhibiting either nuclear envelope repair or DNA damage repair pathways alone did not reduce cell viability, but inhibition of both nuclear envelope and DNA repair substantially increased cell death after NE rupture.

The integration of our migration devices with high resolution time-lapse imaging provides a powerful new approach to study intracellular mechanics and dynamics in a variety of physiologically relevant applications, ranging from cancer cell invasion to immune cell recruitment.

References:
Microfluidics for Modeling Biological Flows in Breast Tumor Cell Invasion

CNF Project Number: 2068-11
Principal Investigator: Mingming Wu
User: Yu Ling Huang

Affiliation: Department of Biological and Environmental Engineering, Cornell University
Primary Source of Research Funding: National Cancer Institute
Contact: mw272@cornell.edu, yh486@cornell.edu
Website: http://biofluidics.bee.cornell.edu/
Primary CNF Tools Used: Karl Suss MA/BA 6 aligner, Unaxis 770, Anatech Resist Strip, MVD100, Heidelberg DWL2000

Abstract:
Cancer metastasis is a physical process where tumor cells break away from the primary tumor, invade through the interstitial extracellular matrix, enter and leave the vascular vessels, and establish a secondary tumor at a distant organ. Increasing evidences have demonstrated that the tumor microenvironment critically regulates tumor cell invasion. Microfluidic platforms are a useful tool in modeling the tumor microenvironment because they are compatible with microscopes and allow for dynamical imaging of cell migration. The unique microsized channel features of microfluidics allow us to model biological flows precisely within a tumor microenvironment, including intramural flow through blood vessels and interstitial flow through the interstitial extracellular matrix. We developed and fabricated a microfluidic device that enables us to mimic both intramural flow and interstitial flow for studying their roles on breast tumor cell invasion.

Summary of Research:
The goal of designing the microfluidic device is to simultaneously model intramural flow through engineered vessels and interstitial flow through three-dimensional tumor embedded extracellular matrices. To achieve this goal, we designed a microfluidic device with five parallel cell channels for endothelial tube formation and one horizontal channel for interstitial flow (Figure 1).

A key feature of the device is its use of microsized contact lines placed in between each two neighboring cell channels. The purpose of the contact lines was to confine tumor cells embedded collagen within a desired channel, and endothelial cells (EC) can be seeded and formed a hollow tube surrounding by collagen (Figure 2 as an example). The advantages of the five-channel layout over our previous 3-channel device [1,2] are flexibility to grow one or two endothelial tubes and spatial arrangement of tumor cells and endothelial tubes.

The microfluidic device was fabricated using the standard soft lithography technique. First, the silicon master was fabricated using a two-step etching method, and then the microfluidic device was replicated from the silicon master using PDMS stamping.

To fabricate the negative silicon master, the micro contact lines with dimension of 10 µm in width and 5 µm in depth were first etched, followed by a 200 µm deep etch for the cell and horizontal channels. Photoresist S1813 was first spun on a silicon wafer at 3000 rpm for 45 second, and then baked at 115°C for 60 second before exposed to 128.7 mJ/cm² on a contact aligner. After developing the resist with MF-321, the wafer was etched using a Botsch® deep silicon etching method to obtain the 5 µm contact lines.
To make the cell and horizontal channels as a second layer, SPR220­7.0 photoresist was spun on the etched wafer with 2500 rpm for 40 second, and baked at 115°C for 90 second before exposure with dosage of 1.05 J/cm², followed by post baked for another 90 second at 115°C. After resist development with AZ 726MIF, the wafer was etched for 200 µm depth. After resist striping with oxygen plasma for one hour, the wafer was silanized with a single layer of (1H,1H,2H,2H-Perfluorooctyl) trichlorosilane (FOTS) using a vapour deposition method. To prepare the microfluidic device, PDMS at 10:1 ratio was then poured onto the silicon master to replicate a positive feature for our experiments.

Figure 3 illustrates our first step to confine collagen at two side cell channels by the contact lines while endothelial cells were introduced into the middle cell channel. Intramural flow was applied through the EC tube to facilitate the formation of an EC tube.

References:

Figure 2: Schematic of cross sectional view of the microfluidic device (zoom in from dash line in Figure 1). Small rectangles in between channels represent the contact lines (10 µm by 5 µm). Collagen gels are confined in individual channels whereas endothelial tubes formed in the empty channel surrounded by collagen. Intramural flow can be applied through the EC tubes and interstitial flow can be applied through the horizontal channel.

Figure 3: Micrograph of EC tube formation within a channel surrounded by collagen in the two side channels.
Zero-Mode Waveguides on Thin Silicon Nitride Membranes for Efficient Single-Molecule Sequencing

CNF Project Number: 2214-13
Principal Investigator: Prof. Meni Wanunu
User: Dr. Vivek Jadhav

Affiliation: School of Physics, Northeastern University
Primary Source of Research Funding: NIH award No. 1R01 HG009186
Contact: wanunu@neu.edu, v.jadhav@neu.edu
Website: http://www.northeastern.edu/wanunu/
Primary CNF Tools Used: LPCVD CMOS nitride - E4, JEOL 6300, SC4500 odd-hour evaporator, Zeiss Ultra SEM

Abstract:

Single-molecule, real-time (SMRT) DNA sequencing using zero-mode waveguides (ZMWs) offers long reads by polymerase bound DNA template. We demonstrate low-concentration of DNA capture by constructing ZMW on a 50 nm thick silicon nitride membrane and drilling a nanopore (~3-4nm) at the base of the waveguide using TEM. To protect the aluminum from electrochemical reactions, we coat the ZMWs with a thin layer of silicon dioxide using atomic layer deposition. A polymerase-streptavidin bound DNA template is anchored to the exposed biotin at the bottom of a ZMW by applying a voltage bias. DNA polymerase replicates the sample DNA as it incorporates new fluorescently-labeled phospholinked nucleotides, emitting a burst of light before the phosphate is cleaved off, giving a color sequence that corresponds to the DNA sequence.

Summary of Research:

Single-molecule detection at micromolar concentration is achieved by fabricating zero-mode waveguides (ZMWs) with subwavelength holes in a metal film [1]. An essential component of the single molecule, real-time (SMRT) sequencing is the zero-mode waveguide (ZMW), a cylindrical cavity in which the DNA and DNA polymerase molecules are immobilized [1].

A single molecule of DNA template-bound DNA polymerase is immobilized at the bottom of a ZMW, which has excitation confinement in zeptoliter enables detection of an individual fluorescently-labeled phospholinked nucleotides [2]. The ZMW nanostructure are fabricated on a 50 nm thick silicon nitride (deposited using LPCVD CMOS nitride - E4) membranes using electron-beam lithography (JEOL 6300) at CNF (Figure 1). We showed that making ZMW and drilling a 3.5 nm pore at the bottom of the ZMW, the efficiency of molecular loading into these structures could be enhanced by orders of magnitude [3].

We continue to fabricate these devices for our DNA sequencing experiments. Increasing the size of ZMW (~110nm) we can capture large DNA molecule with ease. To protect the aluminum (deposited using SC4500 odd-hour evaporator) from electrochemistry with chloride buffer, which might occur while apply a voltage bias during an experiment, we passivate the aluminum with ~ 13 nm of silicon oxide using atomic layer deposition technique (done outside CNF , Fig. 2).

These ZMW array chips are used for capturing DNA-polymerase complexes. In Figure 3, we see a sample time trace from our DNA-sequencing experiment. Each fluorescent burst corresponds to individual dATP, dTTP, dGTP, dCTP nucleotide being incorporated into a new DNA strand.

References:

2016-2017 Research Accomplishments

Biological Applications

Figure 1: Scanning electron microscopy image of an array of ZMWs on a thin silicon nitride membrane.

Figure 2: Transmission electron micrograph of a ZMW with a nanopore at its center (white circle). The ~13 nm layer of ALD silicon dioxide.

Figure 3: False-color spectrally-resolved fluorescence intensity vs. time data obtained during sequencing experiment. Legend shows the color of each of the dNTPs.
Microfabricated Devices for Cell Organization

CNF Project Number: 2249-13
Principal Investigator: Minglin Ma
User: Wei Song

Affiliation: Department of Biological and Environmental Engineering, Cornell University
Primary Source of Research Funding: American Diabetes Association
Contact: mm826@cornell.edu, ws336@cornell.edu
Website: http://malab.bee.cornell.edu
Primary CNF Tools Used: Heidelberg DWL2000 mask writer, ABM contact aligner, PDMS coating station, SU-8 hotplates

Abstract:
Different types of cells dynamically self-assemble and organize themselves in a spatiotemporal and context-dependent manner [1]. In this study, we report the spatiotemporal dynamics of cell organization of a binary cellular mixture (MDA-MB-231 and MCF10A cells) seeded in microfabricated microwells. The initial seeding ratio of binary cells determined the degree of encapsulation of MCF0A cells by MDA-MB.231 cells. When cells were free to grow, the differential proliferation rate of MDA-MB-231 (low growth rate) and MCF10A cells (high growth rate) resulted in a reversed core (MDA-MB-231)-shell (MCF10A) organization at seeding ratio of 1:1 (MDA-MB-231:MCF10A) and a side-by-side aggregate structure at seeding ratio of 4:1 after long-term culture.

Summary of Research:

Fabrication of Polydimethylsiloxane (PDMS) Microwell. The photomask was prepared using DWL2000 mask writer (Heidelberg Instruments). The silicon wafer was spin-coated with SU-8 2150 photoresist (MicroChem) at 500 rpm for 40 sec and then 2500 rpm for 30 sec. The wafer was covered with the photomask and exposed by a UV photolithography machine (ABM contact aligner) for 32 sec. After being developed and post-baked, the SU-8 master wafer was fabricated. The SU-8 master wafer was then used to create PDMS (Sylgard 184, Dow Corning) mold. A mixture (10:1) of Sylgard 184 silicone elastomer components was casted onto the master wafer and cured at 60°C overnight to prepare a PDMS microwell. Figure 1 is a microscopic image of PDMS microwells.

Formation of Cell Aggregates in PDMS Microwells. PDMS microwells were autoclaved, placed in a 24-well plate, and coated with 1% (w/v) Pluronic® F127 (Sigma) solution before cell seeding to prevent cell attachment on PDMS surface and facilitate formation of cell aggregates. To form cell aggregates, cell suspensions of MDA-MB-231/MCF10A mixture (MDA-MB-231:MCF10A=1:1 and 4:1, total 1.0 x 10^6 cells) were added to each well of 24-well plate with PDMS microwells inside. After four hours of static culture, the cells that were adhered to the interspace between microwells were removed by medium change. The cells that fell into the microwells formed cell aggregates after overnight culture. The cell aggregates were cultured in microwells for nine days. The mixed medium (MDA-MB-231 medium:MCF10A medium=1:1 and 4:1) was changed every two days.

Figure 1: A microscopic image of PDMS microwells. Scale bar: 1000 µm.
Figure 2 is a fluorescent image of cell segregation of MDA-MB-231 (red colour / darker grey) and MCF10A (green colour / lighter grey) cells at 1:1 cell seeding ratio over nine days of culture. Figure 3 is a fluorescent image of cell segregation of MDA-MB-231 (red colour / darker grey) and MCF10A (green colour / lighter grey) cells at 4:1 cell seeding ratio over nine days of culture.

In summary, the initial seeding ratio and cell proliferation have significant effects on the evolution of cell organization of binary cellular mixture over long-term culture. Depending on the initial seeding ratios, the cell organization is either a core-shell (1:1) or side-by-side (4:1) aggregate by the differential proliferation rates of MDA-MB-231 and MCF10A cells.

References:
Electrochemical Detection Array Combining Amperometry and Total Internal Reflection Fluorescence

CNF Project Number: 2260-13  
Principal Investigator: Manfred Lindau  
User: Meng Huang

Affiliation: School of Applied and Engineering Physics, Cornell University  
Primary Source of Research Funding: National Institutes of Health  
Contact: ML95@cornell.edu, mh2236@cornell.edu  
Primary CNF Tools Used: ABM contact aligner, CHA Mark 50 evaporator

Abstract:

Neurotransmitters are released in a quantal event by fusion with membranes. The mechanism of this fusion event remains unknown, but is crucial for molecular manipulation and various kinds of disease. We develop and fabricate an electrochemical detection array capable of combining amperometry measurement and total internal reflection fluorescence (TIRF). Amperometry provides the information for the releasing neurotransmitters from vesicles in the cell while TIRF enables direct visualization of vesicles with appropriate fluorescence labels. The combination of the two methods offers a new way for studying the exocytosis process.

Summary of Research:

Exocytosis is the process where neurotransmitters are released into the extracellular space [1]. The amperometry measurement provides precise details about the released transmitters in a single quantal event. While amperometry has the above-mentioned advantages, it measures the releasing contents reaching the electrodes and cannot directly characterize the releasing mechanisms. The total internal reflection fluorescence (TIRF) can detect the fluorescence signals at the substrate surface to visualize the footprint of the cell with its generated evanescence wave. The combination of the two methods offers the availability of monitoring vesicle releasing events and amperometry spikes simultaneously.

To fully utilize the TIRF technology, the specific site of release must be known to locate the fluorescence signal. Therefore, we developed the electrochemical detection (ECD) array with four electrodes between which a cell can be placed, as shown in Figure 1 [1]. Individual fusion events can be detected amperometrically with ~ 200 nm precision, utilizing a map of random walk simulations while the cell surface can be imaged with TIRF microscopy [3].

Figure 1: The micrograph showing the geometry of the 4-electrode ECD device.

Figure 2: Correlation of amperometry and TIRF imaging for a vesicle release event in chromaffin cells loaded with FFN 511. Top image shows the amperometry recording while the bottom image shows the fluorescence signal from the TIRF microscopy.
A 4-inch, 175 µm thick glass wafer was used for the fabrication of the ECDs. Patterns were transferred onto the wafer through general lithography with NFOL 2020 negative photoresist. The photoresist was spin-coated on the wafer with 3000 rpm for 30s, resulting in a thickness of 2 µm. After the soft bake, alignment and exposure was performed with ABM contact aligner for 7s at 12.14 mJ/s. Following the post exposure bake, the photoresist was developed using 726MIF for 70s. Then 10 nm Ti / 150 nm Pt were deposited on the device using the CHA Mark50 evaporator, followed by a lift off process using 1165. A 2 µm thick SU-8 insulation layer was applied on the ECD using SU-8-2002 and only the tips of the four electrodes were left exposed to the environment.

Fluorescence false neurotransmitter (FFN) 511 was used as the fluorescence marker in the experiment. Bovine chromaffin cells loaded with FFN 511 was plated in the center of the 4-electrode ECD. A fluorescent image of cell pressed on ECD electrode was taken by TIRF microscopy while the release events from vesicles were detected by the electrodes through amperometry. By comparing the random walk simulation and the amperometric spikes of the released neurotransmitters, the precise locations of the specific events could be determined through spatiotemporal correlation. As shown in Figure 2, the TRIF imaging at the specific event location shows a decrease in the intensity, indicating a vesicle loaded with FFN 511 disappearing from the TIRF camera, or releasing its contents to the extracellular space.

References:
Microfluidics for Automated Long-Term High-Resolution Imaging of *C. Elegans* Larval Development

CNF Project Numbers: 2293-14, 2518-17 (REMOTE 155)

Principal Investigators: Eric D. Siggia¹, Shai Shaham²

Users: Wolfgang Keil¹,², Michael Skvarla³

Affiliations: 1. Center for Physics and Biology, The Rockefeller University; 2. Laboratory for Developmental Genetics, The Rockefeller University; 3. Cornell NanoScale Science & Technology Facility (CNF)

Primary Source of Research Funding: National Science Foundation

Contact: siggiae@rockefeller.edu, shaham@rockefeller.edu, wkeil@rockefeller.edu

Primary CNF Tools Used: Heidelberg DWL2000, SÜSS MA6-BA6, P10 profilometer, Aura 1000, MVD 100, Unaxis 770

Abstract:

Long-term studies of *Caenorhabditis elegans* (*C. elegans*) larval development traditionally require tedious manual observations because larvae must move to develop, and existing immobilization techniques either perturb development or are unsuited for young larvae. In our project, we developed a simple microfluidic device to image development of *C. elegans* larvae at high spatiotemporal resolution from hatching to adulthood (~3 days). Ten animals, each confined by a circular array of posts, 4.88 µm apart, in a 400 µm diameter chamber, can be periodically immobilized by compression to allow high-quality imaging of even weak fluorescence signals. For the first time, this allows visualization and quantification of developmental processes such as neural arborization, cell divisions, transdifferentiation, and cell death in a feeding, moving, and growing animal. Our technique opens the door to quantitative analysis of time-dependent phenomena governing cellular behavior during *C. elegans* larval development.

Summary of Research:

The nematode *C. elegans* is optically transparent, exhibits an invariant cell lineage, and can be functionally probed using powerful genetic tools, making it a versatile setting for revealing principles of metazoan development. However, long-term high-resolution *in vivo* imaging of *C. elegans* development has been accessible only in embryos, not for developing larvae [1]. The goal of the CNF projects 229314 and 251817/REMOTE 155 was to close this gap by developing a microfluidic device for fast, reliable, reversible, repeatable, and non-damaging immobilization of *C. elegans* larvae at any given post-embryonic stage.

The main work at the CNF involved developing and optimizing the micro-chamber in which the animals would be kept and imaged throughout their post-embryonic development. We tested dozens of chamber sizes and layout geometries and compared them with respect to ease of animal loading, reliability of animal immobilization, and their ability to confine animals of all larval stages. The latter proved to be most challenging. Very young *C. elegans* larvae are 15 µm wide and 250 µm long, yet proved to be flexible enough to squeeze through 5 µm channels. Late larval stage animals and adults are much larger, typically 30-50 µm wide and 800-1000 µm long. Thus, channels to confine animals had to be smaller than 5 µm while at the same time 20-30 µm high.

Previous *C. elegans* microfluidic devices were fabricated by casting and curing PDMS on molds obtained by photolithography on silicon wafers, mostly using photosensitive epoxy (negative photoresist), e.g. ref. [2]. However, durable features of the required dimensions allowing repeated casts could not be easily accommodated by these techniques. The CNF staff thus suggested deep-reactive ion etching (DRIE) to fabricate the molds. This resulted in precise and durable high-aspect ratio features. The final layout of the two-layer microfluidics device consists of ten 400 µm diameter circular chambers, each formed by an array of posts, 4.88 µm apart (Figure 1, [3]).

Worms are immobilized by a two-step procedure: First, we gradually apply increasing negative pressure to the outlet of the flow layer (Figure 1C, left). This elicits increasing flow toward the outlet, pushing animals to the side of the chamber. At the same time, the negative pressure in the compression layer chamber is gradually released back to ambient pressure. Second, we
gradually increase the pressure applied to the compression layer, deflecting the PDMS membrane toward the flow layer below (Figure 1C, right). The deflected PDMS membrane restricts the animal’s movement by pushing it toward the side of the chamber (Figures 1C, right and 1D). Reliable immobilization is achieved within 45-60s. A manuscript describing the technology and several applications has been published in 2017 in Developmental Cell [3].

In collaboration with several labs inside and outside of Rockefeller University, we are currently using our method to study a variety of cellular phenomena, ranging from stochastic cell fate decisions, neurodevelopment to cell death and degradation. We expect the microfluidics setup developed in the course of this CNF project to be highly relevant and extensively applied by a large scientific community and we are in contact with several companies in order to transform our technology into a commercially available platform that could be used by hundreds of *C. elegans* laboratories around the world.

**References:**


Evaluating and Improving a Novel Bioreactor for Mammalian Cell Culture

CNF Project Number: 2298-14
Principal Investigators: John C. March, Cait M. Costello
Users: Rohan Acharya, Marek Andrzej Kwasnica

Affiliation: Department of Biological Engineering, Cornell University
Primary Sources of Research Funding: National Institutes for Health, National Science Foundation
Contact: jcm224@cornell.edu,cmc483@cornell.edu,rba49@cornell.edu, mak436@cornell.edu
Primary CNF Tools Used: ObJet30 Pro 3D printer, Labcoter-2 parylene deposition system

Abstract:
To better study complex biological phenomena and interactions of the small intestine, new cell culture systems must be devised that can better mimic the gastrointestinal microenvironment than the standard Petri® dish. To this end, we have evaluated an in vitro modeling platform that incorporates a three-dimensional growth scaffold for the cells and fluid flow induced mechanical shear stress that simulate the villi of the intestine and the migratory motor complex respectively. The goal of this project was to evaluate the effect of the periodic fluid shear stress on the proliferation, differentiation, and mucus production of Caco-2 cells grown in the bioreactors compared to standard cell culture dish. Additionally, we made design improvements to simplify the closing mechanism of the bioreactor and reduce the potential for leakage and contamination.

Summary of Research:
The small intestine is a long, tubed-shaped organ that functions in nutrient absorption. In the inmost layer of the mucosa, surrounding the lumen, there is a layer of finger-like projections, called villi, that serve to increase the available surface area for adsorption and create a gradient of shear stresses during passage of food and fluids through the gut. This gradient provides a variety of environments for our cells and bacteria to live and grow, that is called the microbiome. As diagnoses of gastrointestinal and metabolic diseases such as irritable bowel syndrome (IBS) and diabetes are on the rise, there is an increased interest in studying the human microbiome. To better study interactions between bacteria and human cells, a culture platform that supports both cells and simulates the periodic stresses of digestion is needed. Our group sought to evaluate the effects of periodic fluid flow on the differentiation, mucus production, and protein expression of cells lining in the mucosal layer, using Caco-2 cells as a model organism.

Using the ObJet30 Pro 3D printer, and Parylene coater, we created bioreactors that would house a porous polyethylene vinyl acetate scaffold (PEVA) that simulates the mucosal layer. The reactor provides two chambers, separated by the scaffold, to simulate the lumen and the basal layers of the small intestine, seen in Figure 1 and in Figure 2 (a later model of reactor). Fluid was pumped over the cells in the upper chamber in 10 minute intervals, followed by 90 minute periods of no flow, to simulate the median rate of active contractions of the migratory motor complex (rhythms of contractions that move food along the gastrointestinal tract) during rest.

We found that the fluid shear stress gradient, seen in Figure 3, created a differentiation gradient for the Caco-2 cells. Younger proliferating stem cells clustered around the base of the villi in areas of low shear stress, while more mature differentiated cells along the villi in areas of exposed to more shear stress and more nutrients. Near the tips of the villi, the oldest cells would die and be sloughed off. Differentiation was determined by expression of alkaline phosphatase, an enzymatic component of microvilli that seek out nutrients in mature cells, and mucus production, associated with younger cells. We also found a twofold increase in expression of the sugar transport enzymes SGLT-1 and Glut-2, as well as a fourfold increase in glucose transport (Figure 4).

The modified bioreactor, seen in Figure 2, proved to be a worthy step in providing an intestinal model that was a better engineered product. While there was some initial leaking of these, a further experimental modification allowed for a better product. Any leaking
in early experiments was shown to result from a lack of vacuum grease or glue and quickly fixed for future experiments.

Further experiments exhibited the accuracy of the resulting culture. Change in mucus production when flow was introduced was drastic and mimicked intestines that did not have inflammation problems. The introduction of a pathogen, PA01, was a good example of changing the microbial environment of the system. The reactor also showed that it could handle multiple bacteria, and accurately display the relationship between them. These experiments provided a good precursor to other possible experiments that could be done with the reactors.

References:
Synthetic Small Intestines

CNF Project Number: 2298-14
Principal Investigator: John C. March
User: Cait M. Costello

Affiliation: Department of Biological and Environmental Engineering, Cornell University
Primary Source of Research Funding: NIH
Contact: jcm224@cornell.edu, cmc483@cornell.edu
Primary CNF Tools Used: ObJet30 Pro 3D printer, Labcoter-2 parylene deposition system

Abstract:
The development of artificial small intestines that realistically mimic in vivo systems will enable improvement of our understanding of the human gut and its impact on human health. In this work, we demonstrate the importance of gut surface topography and fluid flow dynamics, which are shown to impact epithelial cell growth, proliferation and intestinal cell function. We have constructed a small intestinal bioreactor using three-dimensional (3-D) printing and polymeric scaffolds that mimic the 3-D topography of the intestine and its fluid flow. Our results indicate that transepithelial electrical resistance (TEER) measurements, which are typically high in static 2-D Transwell® apparatuses, is lower in the presence of liquid sheer and 3-D topography compared to a flat scaffold and static conditions. There was also increased cell proliferation and discovered localized regions of elevated apoptosis, specifically at the tips of the villi, where there is highest sheer. Similarly, glucose was actively transported (as opposed to passive) and at higher rates under flow.

Summary of Research:
Transwells have long been used as the standard in vitro culture method for studies of intestinal absorption, as they provide both an apical and basolateral spaces to simulate the gut-blood-barrier and enable both active and passive transport of drugs and nutrients. However, intestinal cells seeded onto flat supports exhibit markedly different phenotypes to cells in vivo [1], partly due to the poor representation of the 3-D extracellular microenvironments. We have previously shown that recreating the topography of the small intestine with biocompatible collagen or poly-lactic-glycolic acid (PLGA) scaffolds populated with accurately sized villi can lead to improved differentiation and paracellular permeability of Caco-2 monolayers along the villus axis [2]. Cells in a 3-D villus environment experience different nutrient gradients (including oxygen) than cells grown on flat surfaces [3], which can affect their physiology including differentiation. Also, spatial microstructure can influence cell-cell junctions, cell-matrix contacts and molecular communication [4].

In addition to the complex topographical and cellular environment, the human intestine exhibits mechanically active peristaltic motions and fluid flow after ingestion that guide the food bolus down the intestine, facilitating absorption. Specifically, the stomach and small intestine create shear stresses via the hormone-mediated migrating-motor-complex (MMC), an inter-digestive pattern that is dominated by cycles of stasis and short, high-pressure bursts of peristaltic motions, that serve to propel small particles and microorganisms towards the colon [5]. Even during fasting, there is an intermittent flow that exposes the intestinal epithelia to shear stresses. Cyclic strain and shear stress have been shown to modulate signaling pathways in the gut, including those mediated by mechano-sensing β1 and β3 integrins. Importantly, these activate the Rac1 and ERK-signaling pathways (among others) and the downstream wnt/β-catenin pathways which control cellular proliferation and differentiation [6].

What is missing is a device that provides both the accurately-sized villus topography and fluid flow to improve study of intestinal absorption, drug delivery, and intestinal barrier function. Towards this aim, we developed a 3-D printed bioreactor that can both contain villus scaffolds and also create separation of the apical and basolateral spaces in a manner (Figure 1). We cultivated cells in these systems for > 3 weeks, and found site-specific expression profiles of cell differentiation and apoptosis along the crypt-villus axis that are more similar to in vivo than Transwell culture or static 3-D
models (Figure 2). In addition, we evaluated intestinal function by measuring the rate of glucose absorption through the epithelial monolayers, finding significantly more uptake than in static systems. Importantly, levels reached are consistent with estimates for physiological conditions.

We anticipate that such small intestinal bioreactors will be useful for future studies of intestinal function, including high throughput drug absorption profiling and studies of bacteria-host interactions.

References:

Figure 1: Bioreactor setup. A porous PEVA scaffold (A) was seeded with Caco-2 cells and fitted into the assembled 3-D printed bioreactor vessel (B) using the o-rings to seal it in place. After connection to influent and effluent tubing and the peristaltic pump, the device is placed inside the CO₂ incubator for 3-5 weeks (C). TEER measurements were taken daily by connecting the silver wires to chopstick electrodes and then to a voltohmmeter.

Figure 2: 3-D confocal rendering of PEVA scaffolds after growth of Caco-2 cells in the bioreactors and under static conditions. Images were taken at 20X magnification and stained for proliferation with EdU staining [green] (A1-2) and apoptosis with Tunel Assay [green] (B1-2). Caco-2 monolayers grown in the bioreactors have more proliferating cells and the apoptotic cells are localized to the tips of the villi where the highest shear is. Conversely, the static monolayers have less proliferating cells near the base and a large increase in apoptotic cells.
Separation of Submicron Particles in a Surface Acoustic Wave Acoustofluidic Resonator

CNF Project Number: 2349-15
Principal Investigator: Brian J. Kirby
User: Prateek Sehgal

Affiliation: Sibley School of Mechanical and Aerospace Engineering, Cornell University
Primary Source of Research Funding: Center on the Physics of Cancer Metabolism (Award number 1U54CA210184-01 from the National Cancer Institute)
Contact: kirby@cornell.edu, ps824@cornell.edu
Website: http://blogs.cornell.edu/kirbyresearch/prateek-sehgal/
Primary CNF Tools Used: SC4500 odd-hour evaporator, ABM contact aligner, Heidelberg DWL2000

Abstract:
Separation of submicron particles, specifically based on their size, has become ubiquitous to numerous biological and chemical studies. One specific example is the need to separate subpopulations of tumor-derived vesicles — microvesicles ranging from 100-1000 nm and exosomes ranging from 30-100 nm [1]. The current approaches, such as ultracentrifugation and ultrafiltration, fail to usefully isolate the subpopulations of tumor-derived vesicles owing to the fragility and small size of these particles. Acoustophoresis has recently emerged as a label-free and gentle technique to separate biological particles based on their physical properties such as size, density, and compressibility. However, separation of submicron particles has been challenging to date because of the weak acoustic radiation force (ARF) on the particles and the domination of secondary effects, such as acoustic streaming, which inhibits the controlled manipulation of the particles. In this work, we have developed a surface acoustic wave (SAW)-based acoustophoretic device that integrates Fabry-Perot acoustic resonators in a novel microfluidic geometry to effectively separate submicron particles. We present numerical simulations of the device that demonstrates the acoustic field generation in a microfluidic channel, which is used for size-based separation of submicron particles. Finally, we experimentally demonstrate the acoustophoretic separation of 500 nm and 100 nm particles in our device at 6.7-fold higher total flow rate and 3-fold lower power density relative to the previous work [2].

Summary of Research:
Theory. Our device is an assembly of Fabry-Perot SAW resonator and the PDMS microfluidic channel that is bonded directly over the resonator. The SAW resonator consists of one interdigitated transducer (IDT) that is bounded by the Bragg reflectors on both sides, creating the Fabry-Perot modality. The PDMS microfluidic channel is aligned at an orientation angle $\theta$, as shown in Figures 1 and 2.

The standing SAW (SSAW) field, which is generated by each finger-pair of the IDT (Figure 3) and strongest at the center of the IDT, is utilized for submicron particle separation. The Bragg reflectors strengthen the SSAW field on the IDT and create a resonant cavity by reflecting the traveling SAW emanated from both sides of the IDT. The SSAW field couples to the fluid and generate standing acoustic field in the channel, as shown in numerical simulations (Figure 3). The particles suspended in the fluid experience size-dependent ARF in this standing
acoustic field. The orientation of the IDT and the flow field results in size-dependent migration trajectories of the particles — the large particles migrate parallel to the IDT and the small particles migrate parallel to the flow field. This differential migration of particles results in the size-based separation of submicron particles in our device. Importantly, the Fabry-Perot modality enhances the overall ARF on the particles and the SAW-based excitation of high frequency waves minimizes the effect of Rayleigh acoustic streaming on the particles. These effects together enable the manipulation of submicron particles in our system.

**Methods.** The Cr-Au electrodes (10/400 nm, 24 finger-pairs) for a 80 µm SAW wavelength along with 12 reflector strips on each side of the IDT are patterned on 500-µm-thick 128° Y-X cut lithium niobate (LiNbO3) using the standard photolithography and lift-off techniques. Microfluidic channel of height 50 µm are cast in PDMS from SU-8 molds. The PDMS channel and the IDT are bonded at θ = 80° by aligning the alignment marks in each component. The device is characterized with 500 nm fluorescent polystyrene beads injected in the middle channel with DI water as sheath (buffer) fluid. The fluorescent images are acquired using a CCD camera and an upright microscope.

**Experiments.** Figure 4 shows the migration of 500 nm (yellow) and 100 nm particles (blue) in our device. Both the particles migrate parallel to the flow field, at an angle to the electrodes, before and after the IDT region. The 500 nm particles migrate parallel to the electrodes over the IDT region, whereas the trajectory of the 100 nm particles is still dominated by the flow field over the IDT region. A distinct separation is seen at the exit of the IDT region (right side of the image). The formation of bands by the 500 nm particles is consistent with the acoustic focusing of these particles at the pressure nodes. The separation of two populations is achieved at the power density and the total flow rate of 0.12 W/mm² and 12.2 ul/min, respectively. The power density is 3-fold lower and the total flow rate is 6.7-fold higher than the previously reported parameters for the separation of 500nm/300nm particles [2]. This enhanced acoustic effect in our device is attributed to the Fabry-Perot modality of our system. These results demonstrate the proof of concept of our device to separate submicron biological particles. Because the particles are levitated in the acoustic field over the IDT, we expect that the acoustophoretic separation modality of our device is suitable to separate subpopulations of tumor-derived vesicles without damaging these vesicles.

**References:**

High Confinement and Low Loss Si₃N₄ Waveguides for Miniaturizing Optical Coherence Tomography

CNF Project Number: 2364-15
Principal Investigator: Michal Lipson
Users: Xingchen Ji, Aseema Mohanty

Affiliations: 1. Department of Electrical Engineering, Columbia University, New York, NY 10027; 2. School of Electrical and Computer Engineering, Cornell University, Ithaca, NY 14853
Primary Source of Research Funding: Defense Advanced Research Projects Agency
Contact: ML3745@columbia.edu, xj53@cornell.edu, am2353@cornell.edu
Primary CNF Tools Used: LPCVD, e-beam lithography, Oxford 100 etcher, AJA sputter deposition

Abstract:
We show high confinement thermally tunable, low loss Si₃N₄ waveguides that are 40 cm long. We show that this platform can enable the miniaturization of traditionally bulky active optical coherence tomography (OCT) components.

Summary of Research:
Optical coherence tomography (OCT) has revolutionized fields such as ophthalmology and dermatology with noninvasive real-time micron scale resolution imaging. OCT is an interferometric imaging technique capable of providing high-resolution, cross-sectional and three-dimensional images with micrometer-scale axial resolution at depths above a millimeter [1,2].

The interferometer in commonly used OCT systems is based on fiber or individual free space optical components that limit the stability, cost and size of the systems. Integrated photonics could miniaturize the interferometer by providing a compact, low-loss and tunable optical path. This would not only improve the stability of the interferometric detection but could also significantly reduce the size and cost of the whole system [3]. Recently, interferometers in material platforms such as silicon on insulator, silicon oxynitride and thin silicon nitride (Si₃N₄) have been demonstrated [4-6]. However, all of these studies use an external free-standing reference arm to compensate the optical path length that limits the degree of miniaturization. In order to have a sufficiently long on-chip reference arm, loss becomes a critical issue.

The challenge in integrating the reference arm on-chip, one of the key components needed for miniaturization, is the tradeoff between the need for low loss and for a long tunable arm. While low loss is usually achieved in glass, this platform does not present a tunability mechanism and requires a much larger bending radius. Waveguides consisting of SiO₂ surrounded by a thin layer of Si₃N₄ designed to reduce bending radius have been demonstrated as an on-chip reference arm [7]; however, the minimum bend radius achieved is still a few millimeters and due to the weak light confinement in Si₃N₄, it does not provide enough thermo-optic interaction for tuning.

Here we break the traditional trade-off between loss and tunability using low loss Si₃N₄ waveguide with high confinement for efficient thermo-optic effect. The low loss is achieved by using a new etching process where the polymer residue from the etching process left on the sidewalls is reduced [8]. E-beam lithography is commonly used in photonic device fabrication. For a sufficiently long waveguide, typically multiple e-beam lithography fields have to be crossed. Due to field shifts and stage instability, stitching happens at the field boundary that dramatically increases propagation loss in the waveguide. By adiabatically tapering the size of the waveguide to a wider width at these stitching boundaries, we significantly decrease the propagation loss due to the stitching. The thermo-optic coefficient of Si₃N₄ is about 2.45 ± 0.09 × 10⁻⁵ RIU/°C [9], micro-heaters can be integrated with Si₃N₄ structures, which gives us tunability on the centimeter scale.

We fabricate Si₃N₄ waveguides that are tens of centimeters long with losses as low as 0.17 ± 0.01 dB/cm. We compare the 40 cm long waveguide that crosses multiple different e-beam fields with shorter waveguides that have no field crossing. The propagation loss fit is still linear, which shows that
our novel adiabatic taper design helps to reduce the stitching loss. The propagation loss can be further reduced by applying process described in [8].

We conduct OCT imaging using the thermally tunable Si₃N₄ waveguide as the reference arm of interferometer. A spectral-domain OCT system at 1300 nm was employed to generate the cross-sectional images. As shown in the figure, the quality of the image remains almost the same, which means our on-chip Si₃N₄ waveguide can be used to replace fiber reference arm without sacrificing image quality.

Utilizing low loss high confinement thermally tunable Si₃N₄ waveguide and a novel adiabatic taper design, we are able to fabricate sufficiently long on-chip reference arm for OCT. Integrated photonics has the ability to miniaturize the size and cost of the OCT systems, as well as to increase the stability and provide centimeter scale tunability, which leads to a fully integrated OCT system on chip.

References:
Handheld Chem/Biosensor Combining Metasurfaces and Engineered Sensor Proteins to Enhance Surface Plasmon Resonance (SPR)

CNF Project Number: 2430-16
Principal Investigator and User: Lori Lepak

Affiliation: Phoebus Optoelectronics, LLC
Primary Source of Research Funding: Department of Defense
Contact: llepak@phoebusopto.com
Website: www.phoebusopto.com
Primary CNF Tools Used: Heidelberg DWL2000, ASML DUV stepper, SC4500 evaporator, Zeiss SEM

Abstract:
Since 2003, Phoebus Optoelectronics has enabled custom R&D solutions in the fields of Metamaterials, Plasmonics, Antennas, and Sensors. We work closely with our customers throughout device development, from product realization to small volume manufacturing. Our R&D portfolio spans the spectral ranges of visible light, infrared, terahertz, and microwave radiation, for applications in high resolution infrared imaging systems, wavelength and polarization filtering, tunable optical components, beam forming and steering, solar cells and renewable energy devices, and chemical and biological toxin sensors. Our agile team makes extensive use of the resources at the CNF for our nano/micro fabrication and testing, to provide cost efficiency and rapid turnaround.

In the present report, we discuss recent efforts to develop a chem/bio toxin detection system, which provides the state-of-the-art sensitivity of a typical benchtop system with the superior SWaP performance of a handheld system. Our surface plasmon resonance (SPR)-based sensor is expected to be capable of detecting ng/mL concentrations of selected toxins in under five minutes.

Summary of Research:
SPR is a highly sensitive, label-free optical detection technique, whose underlying physics is illustrated in reflection mode in Figure 1. A laser passes through a prism, at an incident angle \( \theta \), on a gold film that is in contact with an analyte solution on its opposite side. The illumination produces an evanescent wave (surface plasmon), which significantly reduces the reflectance at a resonant angle. This resonant angle is strongly dependent on the local refractive index, within a few tens of nanometers of the gold surface, and thus is extremely sensitive to enzyme-substrate or antibody-antigen binding events near the surface. The resonance is independent of the geometric configuration of the optical elements (see [8] for mathematical derivation), such that these results also apply to devices that operate in transmission mode.

As illustrated in Figure 2, Phoebus has combined two recently developed technologies to enable an SPR sensor system that provides enhanced sensitivity at lower SWaP, relative to technologies currently on the market. First, Phoebus detects toxins using engineered intrinsically disordered proteins (IDP’s), designed to undergo extreme conformational changes upon binding target (b) Gold metasurface, patterned to maximize transmission at SPR resonant wavelength. Adapted from ref. [8].
undergo an exceptionally large conformational change upon binding their specific target. This conformation change increases the density of the protein layer, thereby locally increasing the effective refractive index, which in turn enhances the SPR signal by a factor of 100-1000x competing systems.

Second, Phoebus uses the resources of the CNF to fabricate plasmonic chips patterned with a metamaterial surface to enable extraordinary optical transmission (EOT), a phenomenon unique to metastructures in which light is transmitted through apertures much smaller than the incident wavelength, at anomalously large intensities relative to the predictions of conventional aperture theory. EOT was first observed by T.W. Ebbesen in 1998 [1]. Since its founding in 2003, Phoebus has successfully harnessed EOT by incorporating metasurfaces into devices used to perform light filtering [2-3], photon sorting [4-5], polarimetric detection [6], high speed optical detection [7], and most recently, in our SPR plasmonic sensor chips [8].

These two innovations are combined by attaching the engineered IDP’s to the patterned gold metasurface using standard thiol-based attachment chemistry, to make a disposable sensor chip. As shown in Figure 3, this chip is inserted into the complete handheld 3D printed module. All of the optical elements are already assembled in-line as indicated, for a transmission based detection system. Except for Phoebus’s disposable sensor chip, all of the optical components are inexpensively commercially available, which helps to make our overall system a highly cost-effective toxin sensing solution.

Our first-generation metasurface chips, imaged in the SEM in cross section in Figure 4, consist of an array of gold wires, which serve both to bind the designed IDPs and to undergo SPR. To make the chip, we patterned the wires using the ASML DUV stepper, evaporated Cr/Au, and performed a liftoff. This process is capable of consistently producing lines down to ~ 200 nm wide, with smooth enough sidewalls for an operable optical device.

References:
Scalable Sensor Array Platform for Analysis of Quantal Transmitter Release Events

CNF Project Number: 2460-16
Principal Investigator: Manfred Lindau
User: Meng Huang

Affiliation: School of Applied and Engineering Physics, Cornell University
Primary Source of Research Funding: National Institutes of Health
Contact: ML95@cornell.edu, mh2236@cornell.edu
Primary CNF Tools Used: ABM contact aligner, Unaxis 770 Deep Si etcher, AJA sputter deposition system

Abstract:
Neurontransmitters are released in a quantal event by fusion with membranes. We develop and fabricate a CMOS sensor array capable of parallel electrochemical detection of vesicle release events from chromaffin cells. To enable amperometry measurement, polarizable platinum electrodes are deposited on the Al/Cu metal contact on the CMOS chip by sputtering. SU-8 insulation layer is also applied to protect the surface structure of the chip and avoid incomplete coverage of the metal contact by shifting the position of the electrodes as well as form deep wells to trap cells. A silicon wafer with deep etched wells is used as holder for the CMOS chips for better handling and pattern transfer.

Summary of Research:
Neurontransmitters are released into the extracellular space in a process known as exocytosis [1]. The amperometry measurement provides precise details about the released transmitters in a single quantal event. However, amperometric spikes vary from cell to cell even under the same condition [2]. Therefore, a large number of measurements for vesicle release events must be performed to achieve a change in the mean value. Here, we present the CMOS IC sensor array capable of parallel amperometry measurement of vesicle release events and the post-fabrication to enable its functionality.

The CMOS chip has a die dimension of around 3 mm × 4 mm. Therefore, direct spin coating of photoresist on the chip will leave a severe side effect and distort the pattern, especially for the viscous SU-8. Here, we fabricate a silicon wafer holder for the chips for better handling and pattern transfer. First, SPR220-7.0 resist is spin coated on the wafer at 3000 rpm for 30s followed by a 90s soft bake. Then, it is exposed using ABM contact aligner to transfer the pattern (exact dimension of the die but with 50 µm margin) on it as a mask layer for etching. After the post exposure bake and development in 726MIF, the wafer is etched using Unaxis 770 deep silicon etcher to make a 250 µm deep well for the chip to fit in.

The CMOS sensor chip is fabricated at MOSIS by On Semiconductor CSF/N. Polarizable electrode materials such as platinum are not offered in this process. Instead, Al/Cu metal contact are deposited to serve as interconnection of the chip. However, amperometry measurement requires polarizable electrodes for low noise current measurement as the oxidation current is usually on the order of pA. Hence, it is necessary to have a post-fabrication process in the CNF clean room to deposit platinum electrodes directly onto the Al/Cu metal contacts for amperometry measurement. AJA sputtering system is used to deposit Ti(60s)/Pt(500s) bilayer with 400w power on the electrode to have a uniform metal film as well as good side wall coverage.

To avoid possible defect such as incomplete coverage of the Pt electrode, a shift electrode strategy is performed to redefine the position and shape of the working electrodes (Figure 1) [4]. The shifted electrodes also enable cell trapping by SU-8 deep wells. The patterned poly(L-lysine) in register with the electrodes will promote cell adhesion, while poly(ethylene glycol) is applied in between wells will resist cell adhesion [5]. Pt electrodes are deposited over the Al/Cu contact, but instead of just covering the contact window, they are extended to cover some part of the overglass. Finally, a 16 µm SU-8 2025 thick layer is fabricated on the surface of the CMOS chip.
Deep wells with 20 µm in diameter are opened by general lithography at the redefined electrode position. The round shape rather than the original square shape of the electrode opening is beneficial for cell trapping (Figure 2). Microcontact printing of poly(L-lysine) and poly(ethylene glycol) will be performed for promotion and resistance of cell adhesion. The product was tested with 350 µM Dopamine solution and the dopamine molecule diffusion can be observed from the current level of each pixel on the chip (Figure 3). Live cell amperometry recordings also show clear and clean amperometric spikes (Figure 4).

The device is able to measure multiple cells in parallel, facilitating the experiment in terms of time and efforts.

**References:**


Droplet- Microfluidic Device for Stem Cell Culture

CNF Project Number: 2461-16
Principal Investigator: Benjamin D. Cosgrove
User: Andrea J. De Micheli

Affiliation: Meinig School of Biomedical Engineering, Cornell University
Primary Sources of Research Funding: Cornell start-up funds, NIH Grant # R00AG042491 (Cosgrove)
Contact: bdc68@cornell.edu, ad689@cornell.edu
Website: http://blogs.cornell.edu/cosgrove/
Primary CNF Tools Used: Heidelberg DWL66FS/2000, SÜSS MJB4, SU-8 hotplates

Abstract:
We are working on a droplet-microfluidic device to generate microscopic beads of poly(ethylene glycol), a biomaterial we use to study the interaction between muscle stem cells and their environment. The device is made from PDMS cast on a SU-8 patterned wafer generated by standard SU-8 photolithography techniques at CNF.

Summary of Research:
Microfluidics have enabled a more high-throughput and comprehensive examination of biological systems. In particular, the interaction between stem cells and their local environment (the niche) can be studied using biomaterial constructs that attempt to recreate physical and biological aspects of the niche. We used a droplet-microfluidic device (designed and built at CNF) to generate hundreds of thousands of beads of the biomaterial poly(ethylene glycol) (PEG) with various physical and biochemical properties. We will be using these ~100 µm PEG beads as artificial microenvironments to screen for muscle stem-cell-niche interactions that are characteristic of muscle physiology. So far, we were able to create PEG beads with a different level of incorporated Laminin and observe myoblast binding in culture.

Figure 1: 100-micron PEG beads coated with the fluorescent (Alexa647) protein laminin (red / light grey). Clusters of myoblasts can be seen adhering to the beads.

Figure 2: Left: SU-8 wafer with patterned structures. Right: A series of eight PDMS microfluidic devices.

Figure 3: Droplet-microfluidic setup for generating PEG beads. Courtesy De Vlaminck lab.
Graphene Film Protein Crystal Mount

CNF Project Number: 2467-16
Principal Investigator: Marian Szebenyi
User: Irina Kriksunov

Affiliations: CLASSE, MacCHESS; Cornell University
Primary Source of Research Funding: National Institutes of Health
Contact: dms35@cornell.edu, ik29@cornell.edu
Website: www.macchess.cornell.edu
Primary CNF Tools Used: Carbon nanotube/graphene furnace, Zeiss Ultra SEM, VersaLaser engraver/cutter tool

Abstract:
Graphene is a novel material with many distinct properties. Graphene is impermeable to gases and transparent to x-rays. The ability of graphene to reduce the x-ray background and maintain the hydration of protein crystals suggests that graphene could be used as a crystal-mounting platform in protein x-ray crystallography. Multiple layer graphene film is used to construct a protein crystal mount, suitable for x-ray protein crystallography experiment.

Summary of Research:
The graphene-on-copper samples were prepared at the Cornell NanoScale Facility using the carbon nanotube/graphene furnace.

A film consisting of multiple graphene layers was grown on both sides of a thin (100 µm) copper foil. A polymethyl methacrylate (PMMA) coating was applied on top of one side as a support for graphene film. The copper substrate was removed by an etching process using ferric chloride (Transene Company, Inc.). The samples were placed in 100 ml ferric chloride solution in a crystallizing dish for 30 minutes. Once the graphene film with PMMA substrate was free of copper, a series of rinsing procedures were performed using high purity DI water. The graphene film was stored floating on water in the crystallizing dish.

X-ray crystallographic data were collected at Cornell High Energy Synchrotron Source F1 and A1 beamlines using both microbeam set up and 100 µm collimator. Trypsin, ferritin and catalase crystals were mounted on graphene film crystal holders. Two types of crystal mounts were constructed using multiple layer graphene film with 0.5 µm thick PMMA backing layer: graphene wrap mount and graphene window chip.

Graphene Wrap Mount. Graphene wrap mount was constructed using MitegenMesh (MiTeGen, LLC) crystal holder and graphene film with a PMMA layer. Graphene film was wrapped around MitegenMesh mount containing multiple crystals [1]. Data collected at room temperature from Trypsin crystals at 1.35 Å (Figure 1).

Figure 1: Graphene wrap mount.

Figure 2: Graphene window chip.
**Graphene Window Chip.** Nine chips with graphene windows were constructed using samples of multiple layer graphene film with PMMA backing. Various polymer materials were used to make chip insertion and frames [2]. Trypsin crystals were deposited to graphene windows, which were sealed afterward during chip assemble.

Multiple sets of data, consisting of 30 frames and 30 degrees in rotation, were collected from trypsin crystals at room temperature. Trypsin crystals diffracted strongly to 1.35 Å at best (Figure 2). Each chip was stored in an individual Petri® dish along with the wet cotton ball to maintain humid environment. In order to assess the diffraction quality of crystals inside the graphene windows, trypsin crystals were exposed to x­rays again in two, five and forty-five days. After being stored for 1.5 month, some of the crystals were still diffracting to about 2Å, suggesting the graphene windows were capable of protecting the crystals from drying out.

**References:**


**Metamaterials for Biosensing**

**CNF Project Number: 2472-16**

**Principal Investigator: Gennady Shvets**

**Users: Shourya Dutta Gupta, Glen Kelp**

**Affiliations:** Applied and Engineering Physics, Cornell University; Department of Physics, University of Texas at Austin

**Primary Source of Research Funding:** Cornell Faculty Development Grant

**Contact:** gs656@cornell.edu, sd789@cornell.edu, gk389@cornell.edu

**Website:** http://shvets.aep.cornell.edu

**Primary CNF Tools Used:** JEOL 9500, CVC SC4500 evaporator, Zeiss Supra SEM, YES asher etcher

**Abstract:**

Non-invasive and non-destructive identification of different cell types allow for early stage diagnosis and lead to more efficacious potential treatment of various human diseases. For example, early stage cancer detection enables many more treatment options and potential cure as compared to detection in the later stage of cancer. In this respect, circulating tumor cells (CTCs) in the blood stream have been shown to be a strong indicator of early stage of various cancers. However, separation, capturing and identification of CTCs still possess significant challenges with regard to their extremely low concentration as well as the inability of traditional methods to characterize them accurately. The problem of capturing and identifying CTCs is undertaken using two different approaches in our lab: (a) isolation of CTCs from blood using dielectrophoresis (DEP), and (b) spectroscopic identification of cells using mid-IR plasmon resonant metasurface sensors.

**Summary of Research:**

Mid-IR spectroscopy is one of the prominent ways of identifying different materials via their fingerprint molecular vibrations. In the past, this has been used for spectroscopically distinguishing cancerous versus non-cancerous tissue. Typically, at least a complete monolayer of cells is required for performing such a characterization. This limitation on the number of cells creates a large hindrance for adapting this technique for the detection of CTCs, due to their inherently low concentration. It has been previously shown by our group that mid-IR spectroscopy performed using plasmon resonant metasurfaces (Figure 1) allows one to enhance the sensitivity of this technique significantly and we used this approach to accurately characterize a single protein layer [1]. The increase in sensitivity arises from the highly enhanced optical electric fields created near the structures. Furthermore, the metasurface only probes a small region close of the cell membrane due to the rapid decay of the enhanced fields away from the metasurface.

To demonstrate that this technique is viable for cell distinction at a few cell levels, we used these metasurfaces for distinguishing between cancerous (RK0) and non-cancerous (CCD841) colon cells.

![Figure 1: SEM micrographs of the plasmonic metasurface sensor. (a) Low magnification image showing the periodic arrangement of plasmonic microstructures. (b) SEM image of a single unit cell of the metasurface. The metasurface is designed to exhibit very high electric fields in the vicinity of the structure. Depicted unit cells are repeated on the substrate plane to form arrays 100 \times 100 \( \mu \text{m} \)^2 in size. These metasurfaces are made of gold and fabricated on CaF₂ substrates using e-beam lithography.](image-url)
A typical image of metasurfaces with cells on them is depicted in Figure 2a, where the darker region indicates the metasurface. Figure 2b shows a set of representative mid-IR spectra from the two different cells that clearly show a large difference. The difference in the spectral features between the two cell types can thus be used for identifying them. Note that the spectra were acquired in the aqueous state, which is generally not the case in most of the studies in literature. Finally, from a device perspective, the whole experiment is performed within a flow chamber enclosing the metasurfaces, which paves the way for automated and rapid identification and characterization of cells. Deposition of cells directly onto the metasurface sensor is improved with the use of dielectrophoresis (DEP).

Since cells act like dielectric particles, a non-uniform AC electric field can be set up around the metasurface using embedded electrodes and a function generator that cause cell movement due to DEP force (proportional to electric field gradient, Figure 3). Attachment of the cells to the sensor surface is further improved by covering the sensor with antibodies. By tuning the electric field frequency, it is also possible to capture specific cells while repelling other kinds of cells in a multi-species cell solution (Figure 3). Separation of different cell types is especially important while working with blood samples that have very low concentration of CTCs. In the case of CTCs, the separation of tumor and blood cells with DEP is very effective, since those cell types have very different dielectric properties and therefore the frequency of the electric field can be chosen such that CTCs move to the sensor while pushing the blood cells away from it.

References:
Retinal Implant Project

CNF Project Numbers: 2504-16, 657-97

Principal Investigator: Douglas Shire, PhD

Users: Marcus Gingerich, Ph.D.1,2,5, Douglas Shire, Ph.D.1,3,4,5


Primary Sources of Research Funding: Louis Stokes Cleveland VA Medical Center; NIH/NIBIB U01EB018873; NIH/NIBIB R01EB022013; Massachusetts Lions Eye Research Fund

Contact: dbs6@cornell.edu, mdg37@cornell.edu

Website: http://www.bostonretinalimplant.org

Primary CNF Tools Used: Heidelberg DWL2000 mask writer, MA6 contact aligner, polyimide YES curing oven, PT72 RIE, SC4500 evaporator, Gamma Spray Coater, Au electroplating station, K & S Au ball bonder, Zeiss SEM, as well as numerous metrology tools

Abstract:

The purpose of the Retinal Implant Project is to restore useful vision to patients who are blind with degenerative retinal diseases. The primary illnesses we hope to treat are retinitis pigmentosa (a primary cause of inherited blindness) and age-related macular degeneration (the leading cause of blindness in the developed world). Both these diseases cause the eventual destruction of the photoreceptor cells — rods and cones — in the retina, leaving intact the ganglion cells that transmit electrical impulses (and hence visual information) to the brain. The ganglion cells may be stimulated, however, with biphasic current pulses from a microfabricated electrode array. Blind surgical volunteers have consistently described visual percepts that resulted from such stimuli, and this has led our team to develop a wireless, implantable retinal prosthesis.

Summary of Research:

The implanted portion of our device consists of power and data secondary receiving coils, and in a sealed titanium (Ti) can a small number of discrete components, and a custom designed integrated circuit (IC), which consists of circuitry for clock and data recovery, current drivers for electrodes in a stimulating electrode array, and a programmable function generator capable of stimulating with a wide range of pulse widths and amplitudes. The current outputs drive high-charge capacity sputtered iridium oxide film (SIROF) stimulating electrodes, which in turn give rise to the visual percepts mentioned above.

CNF-fabricated components of this system have included various proof-of-concept test structures and tools used in the research effort and an integrated combination flexible circuit and stimulating electrode array. Si wafers serve as carriers for these freestanding films during processing. The electrode leads are fabricated inside of ‘sandwiches’ of polyimide and amorphous silicon carbide (SiC), while the SIROF electrodes are reactively sputter-deposited.

Assembly of the intraocular components of the prosthesis is accomplished by flip chip stud bumping of the IC and solder attachment of discrete components onto an internal flexible circuit board that is hermetically sealed into an ultraminiature Ti can. The RF coils are soldered and glued to the integrated external flex-array, which is in turn thermosonically bonded to the hermetic feedthrough of the Ti can. Finally, the thermosonic bonds are protected and insulated with an overmold. An external patient interface unit, will consist of a video camera for capturing images, a digital signal processor, and a radio frequency (RF) transmitter and coil to relay power and data to the implanted device.

Scientific challenges still remain in realizing a chronically implantable retinal prosthesis. While our first-generation device was primarily encapsulated in polymers for short term proof-of-concept implant studies, our second generation system focused on a system that would last many years in vivo. Our efforts have focused on developing a device with 256+ stimulation channels that is still small enough and of
a configuration to be easily implanted in the ocular orbit and continue to function for many years in vivo. Thus, a major effort has been the development of a technological platform to build a robust, hermetically packaged, high-density subretinal visual prosthesis with a lifetime of > 10 years in biological saline that is scalable to hundreds of I/O channels.

Recent efforts in the CNF have been focused on fabricating, testing and redesigning the electrode array (see Figure 1). The project has also been developing and implementing a process to incorporate SU-8-based 3D electrodes (see Figure 2) into a hybrid electrode array to achieve a better interface between the electrode and the target neural cells. Fabrication work at the CNF has included process development required for such high aspect structures including the challenges of lithography with the presence of such extreme topography (see Figure 3). In addition, alternate methods of interconnection have been explored including Au ball stud bumps some examples of which are shown in Figure 4. The latest microfabrication process utilizes numerous CNF tools including the Heidelberg DWL2000 mask writer, MA6 contact aligner, polyimide YES curing oven, PT72 RIE, SC4500 evaporator, Gamma Spray Coater, Au electroplating station, K & S Au ball bonder, Zeiss SEM, as well as numerous metrology tools.

References:

Figure 1: A picture of a single complete electrode array.

Figure 2: An SEM of two thicknesses of SU-8 structures.

Figure 3: An SEM of an SU-8 structure spray-coated with photoresist to protect during processing and a contact-litho patterned opening to the left.

Figure 4: An SEM of Au balls made with the K & S ball bonder to test the feasibility of a stud bump bond interconnection method.
Biosensor Wafer-Level Device Fabrication

CNF Project Number: 2531-17

Principal Investigator: Dr. Carl Boone, Senior Research Scientist

User: Christopher Alpha

Affiliations: 1. FemtoDx, Inc.; 2. Cornell NanoScale Facility

Primary Source of Research Funding: Venture Capital

Contact: carl.boone@femtodx.com, christopher.alpha@gmail.com

Website: www.femtodx.com

Primary CNF Tools Used: AS200 i-line stepper, JEOL 6300, metal evaporator, CVC evaporator, ALD

Abstract:

We are using CNF to make prototype test devices for a point-of-care medical device start-up company. We have just begun work at CNF and have no results at this time.

Summary of Research:

FemtoDx is a biomedical device start-up company developing silicon-based point-of-care diagnostic tools. The technology is based on initial proof-of-concept work done at Boston University using a limited nanofabrication toolset and minimal process, making devices a few at a time. Wafer-scale processing with good cross-wafer uniformity is the goal of the work at the Cornell NanoScale Facility (CNF).

This project at CNF will process 150 mm silicon-on-insulator wafers to create prototype test devices. This will also provide some initial process validation for moving to a foundry. Currently, the project is in the calibration phase, with a full process run expected for late June, 2017. No data or other figures are available at this time.

The patterned Si structure consists of a group of parallel Si nanowires, which will later be chemically modified for molecular detection specificity. Proprietary electrical measurements will be used to measure molecular binding for scientific and diagnostic purposes.

The nanowires are electrically connected through metal electrodes to bonding pads. Building on our prior work, our CNF-produced devices, which will create hundreds of devices with the exact same process flow due to the $6^2$ wafer capabilities, will be able to include tests of sensor sensitivity to geometric factors and designs. (Some other facilities we have considered are limited to $4^2$ wafers. The SOI wafers we have dedicated for this project only are available in $6^2$ and larger.) It will also incorporate new measurement techniques to improve signal to noise ratio. Some devices will have additional microcomponents to perform basic scientific experiments related to the electromechanical response of proteins to electric fields, different environmental temperatures, and the presence of electroosmotic and electrophoretic flow. Such tests are interesting from a purely scientific standpoint, as they can address unanswered questions about protein binding dynamics and electrophoretic processes, plus a technological standpoint, as they give a starting point for further device optimization.

Approximately eight lithography steps (one e-beam, the rest photolithography), plus standard metal and oxide deposition and Si etching, are required for the current process design. We have critical dimensions of 50 nm (for one e-beam step), and 4 µm (for photolithography steps). All steps are standard processing that can be readily converted to CNF’s toolset, as has already been verified through communications with Christopher Alpha. We expect to get one to two fully processed wafers, with more wafers consumed in process development, over the course of the next six months.

This project is funded by FemtoDX and will be performed under the supervision of senior research scientist Dr. Carl Boone, with input from device engineer Carsten Madler and CEO Raj Mohanty.