

ARO/NSF/SRC Technical Exchange Meeting

Cell-Semiconductor Interfaces and Hybrid Semiconductor-Biological Systems

Meeting Date: July 27 & 28, 2016
Meeting Place: Georgia Institute of Technology, Atlanta, GA.
Meeting Organizers: Hua Wang / Georgia Tech
Dr. Joe Qiu / ARO
Dr. Usha Varshney / NSF
Dr. Victor Zhirnov / SRC

Purpose

Recently, there has been an increasing interest in hybrid biological-semiconductor platforms that can leverage both natural/synthetic biological processes and semiconductor technologies (Fig. 1). In such hybrid platforms, living cells and tissues can function as a “Biological Front-End” layer with the cellular biochemical processes serving as an organic interface to the external environment and performing biological sensing, actuation, signal processing, synthesis, and energy harvesting. In parallel, the underlying semiconductor platforms can form a “Semiconductor Back-End” layer for information computation, control, communication, storage, and energy supply. Most importantly, if reliable two-way communication schemes, for both information and energy, are achieved between the “Biological Front-End” and “Semiconductor Back-End” with a high spatiotemporal resolution and massively parallel operations, one can expect that a hybrid biotic-abiotic feedback system can be created.

The hybrid biology-semiconductor systems can be employed in a broad spectrum of critical applications with ground-breaking scientific, economical, and societal impacts. Leveraging the built-in or synthetically programmed cellular machineries and their interactions with semiconductor platforms, these hybrid systems will potentially offer unprecedented capabilities far beyond conventional electronics-only devices. For example, advances in this field could stimulate developments of self-powered Intelligent Sensor Systems that integrate biological sensing functions and energy generation with inorganic information/computation capabilities to enable diverse new applications. Example applications include fast and high-throughput chemical screening for drug discovery, diagnosis and therapy planning

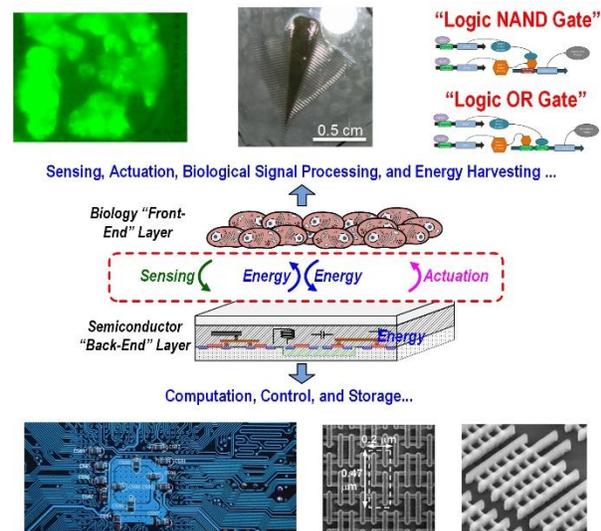


Fig. 1. Hybrid biology-semiconductor platform.

for personalized medicine, detecting chemical and biological agents for defense and environmental needs, and novel microscopic biological actuators or robots.

Current research on such hybrid biological-semiconductor platforms is still in its stage of infancy, and one of the major challenges in such hybrid biology-semiconductor systems lies in the information/energy interface between the cellular “Biological Front-End” layer and the “Semiconductor Back-End” layer. These technological challenges that must be addressed to develop such a hybrid system are daunting and encompass almost every facet of VLSI, nano-electronics, and bioengineering technologies. The meeting participants will examine these essential technologies from the point of view of what near-term advances may be achievable through focused investment in high-risk, high-reward applied research in the area of hybrid bioelectronics microsystems. This meeting will develop future research agendas with a particular focus on the interactions between the nano-electronics and the biological layers, which will enable the next-step scientific explorations on hybrid biological/nano-electronic devices/systems and insure the Nation long-term uncontested technology leadership. This meeting is also designed to identify and roadmap the technology capabilities that are needed to enable the production and deployment of hybrid biological-semiconductor microsystems by 2022.

Format

This is an invitation-only meeting where all the attendees are expected to actively participate. The 1.5-day technical exchange meeting will be constructed to include selected overview presentations and panel discussions. It will encourage and enable interactions by allotting sufficient time for in-depth panel-participant interactions. Participants will be asked to identify technologies and capabilities that may only be achievable through large, focused investment by funding agencies on both fundamental and applied research.

Hybrid Cell-microelectronics Systems for Sensing, Actuating, Synthesis and Computation (Session I)

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Hybrid biological-semiconductor systems will enable a plethora of high-impact applications, including low-cost drug development, chemical screening, environment monitoring, and synthesis of organic/inorganic materials and structures. In the hybrid biological-semiconductor platform, the “Semiconductor Back-End” will perform complex, high-speed, and reprogrammable computations and information storage to enhance the computation in the synthetic “Biological Front-End.” In parallel, the synthetic “Biological Front-End” will offer information pre-processing (sensing and filtering) and post-processing (actuation and synthesis of bio-product) for the “Semiconductor Back-End.” Seamless organic/inorganic signal processing can potentially realize self-regulated operations between “Biological Front-End” and “Semiconductor Back-End,” which may enable fine-grained controls of cellular physiological environment and benefit large-scale cell biology applications. Note that the impact of hybrid cell-microelectronics systems goes well beyond cell biology, since it will also serve as a pivotal technology to improve human health, wellness, and ability. For example, the recent fast growing area of electroceuticals relies on judicious design of cell-electronics interfaces to ensure the right electrical signals are sent into the right cells in a right way. The topics discussed at this session included cell-microelectronics systems for computation, biocompatible organic electronic materials, microfluidic lab-on-the chip platforms, and interfacing nanowires and living cells.

Cell-microelectronics systems for computation

It is becoming increasingly clear that information processing plays a central role in enabling the functionality of biological systems. For example, it has been shown that cell biochemical reactions perform information processing at energy efficiencies that are a few orders of magnitude beyond even advanced semiconductor nanotechnologies of the future, and this is accomplished while achieving a very high information throughput. Therefore, living cells represent alternative models of functional systems that operate efficiently and effectively whose component sizes are on the scale of fractions of a nanometer (Fig. I. 1).

There are astounding similarities between the equations that describe noisy electronic flow in sub-threshold transistors and the equations that describe noisy molecular flow in chemical reactions, both of which obey the laws of thermodynamics. Indeed, chemistry may be viewed as the controlled flow of electrons over relatively short distances while electronics may be viewed as the controlled flow of electrons over relatively long distances. These boundaries are blurring as we reach physical limits where atomic and molecular electronics become chemistry. Therefore, direct sensing, actuation, and computation with molecules in chemically-self-wired fluidic environments will become increasingly

important in advanced man-made structures as it already is in molecular and cell biology. The fundamental laws of noise and thermodynamics set bounds on the energy, time, and space needed to compute at a given speed and precision, which biology is already remarkably close to.

Based on the similarities between electronics and chemistry, it is possible to map circuits between electronic and biological domains in a rigorous fashion^{1,2}. For example, logarithmic analog computation in living cells has been engineered with less than three transcription factors, almost two orders of magnitude more efficient than prior digital approaches, to build a ‘biomolecular slide rule’.

Highly computationally intensive noisy DNA-protein and protein-protein networks can be rapidly simulated in mixed-signal supercomputing chips that naturally capture their noisiness, dynamics, and loading interactions at lightning-fast speeds.

Hybrid cell-microelectronics systems could enable extremely low-energy and high-performance analog and digital computing. Possible first port of entry – point-of-care biomedical computing architectures¹.

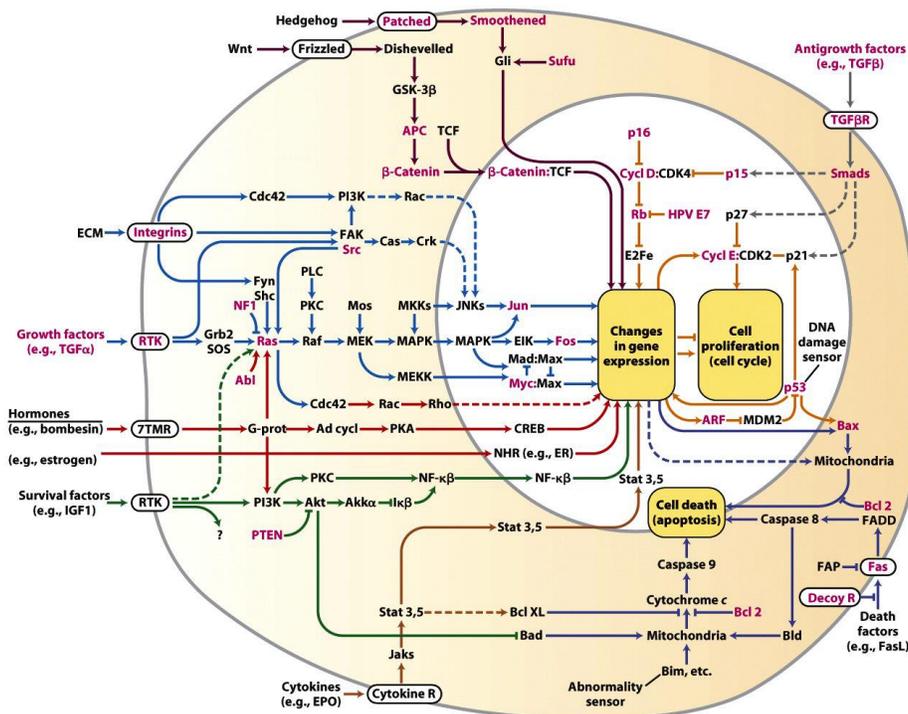


Figure 25-15
Molecular Cell Biology, Sixth Edition
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Fig. I. 1. Living cell as an information processing system¹

Biocompatible organic electronic materials

Biocompatible organic materials are likely to play an important role in hybrid electronic-biological systems. Recently, electronically and ionically conducting polymers were used in an implantable device for local electrically controlled delivery of therapeutics in a living, awake, and freely moving animal ,

(Fig. 1. 2). The conducting polymers form organic electronic ion pump allowing electronic pulses to be transduced into biological signals, in the form of ionic and molecular fluxes, which provide a new way of interfacing biology with electronics. Devices were implanted onto the spinal cord of rats, and local delivery of the inhibitory neurotransmitter γ -aminobutyric acid (GABA) was initiated. The part of the device being inserted subarachnoidally onto the dorsal aspect of the spinal cord was 1.2 mm wide and about 6 cm long. Highly localized delivery resulted in a significant decrease in pain response with low dosage (less than 1% of the amount typically used in intrathecal administration) and no observable side effects. This work reported the first use of an implantable organic electronic device for therapeutic purposes.

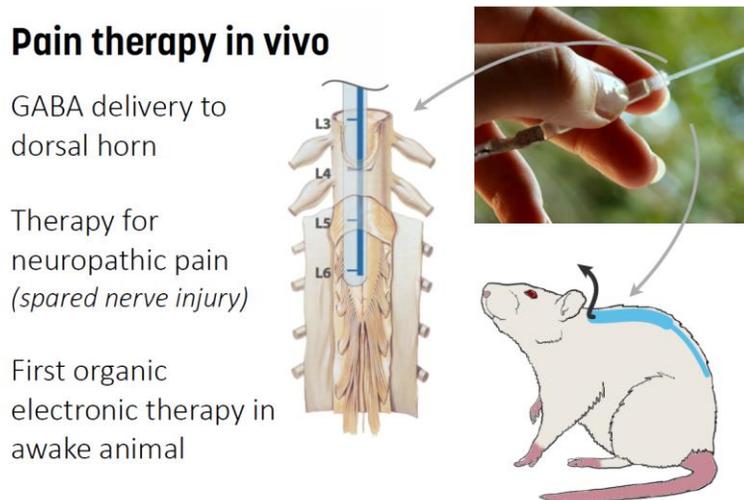


Fig. 1. 2. Organic electronic pain therapy *in vivo*

While the majority of current efforts in bioelectronic interfaces use animal cells, there are very promising opportunities in making bioelectronic interfaces with plants^{3,5}. Plant cell bioelectronic research doesn't have regulatory constraint as in some cases of animal cells. The roots, stems, leaves, and vascular circuitry of higher plants are responsible for conveying chemical signals that regulate growth and functions. This can be viewed as analogous to the contacts, interconnections, devices, and wires of discrete and integrated electronic circuits. Analog and digital organic electronic circuits and devices can be formed in living plants. Possible applications of integrated and distributed electronics in plants, include precision recording and regulation of physiology (e.g., electronic control of root growth rate), energy harvesting from photosynthesis, alternatives to genetic modification for plant optimization, etc.

Experiments with *Rosa floribunda* (garden rose) demonstrated possibilities for creating electrically conducting channels by introducing and dispensing artificial electroactive materials into xylem (a transport tissue in plants). Electrically conducting polymer PEDOT-S:H was used to form extended continuous wires along the xylem channels. These long-range conducting PEDOT-S:H xylem wires, surrounded with cellular domains including confined electrolytic compartments, were shown to act as in situ organic electrochemical transistors. More complex xylem-templated circuits, namely, xylem logic has also been demonstrated^{3,5} (Fig. 1. 3).

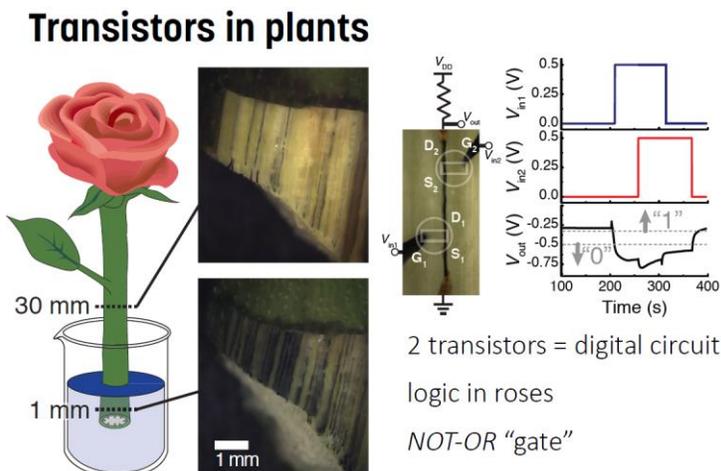


Fig. I. 3. Organic electronic pain therapy *in vivo*

Conductive polymers were also introduced in rose leaves. Vacuum infiltration was used to deposit PEDOT:PSS, combined with nanofibrillar cellulose (PEDOT:PSS–NFC), into the apoplast of rose leaves. PEDOT:PSS–NFC is a conformable, self-supporting, and self-organized electrode system that combines high electronic and ionic conductivity. The result was a leaf composed of a two-dimensional network of compartments filled with the electronic-ionic PEDOT:PSS–NFC electrode material. This system demonstrated a clear electrochromic effect when a bias was applied to the leaf.

In-vivo implantable conducting polymers can become an enabling technology for future electronic plant technologies. The concept e-Plant has been proposed for feedback-regulated control of plant physiology, possibly serving as a complement to existing molecular genetic techniques used in plant science and agriculture^{3,5}. Also, distributed polymer-based conducting wires and electrodes along the stems and roots and in the leaves are preludes to electrochemical fuel cells, supercapacitors, and storage systems that convert sugar produced from photosynthesis into electricity, *in vivo*³.

Microfluidic lab-on-the chip platforms

Portable platforms using microfluidic chips is a foundational technology for interfacing biological matter with electronics. An important driver for development of such platforms is precision diagnostics and therapeutics using many clinically relevant indicators (Fig. I. 4), for example the number of different types of blood cells, microRNA, viral DNA, specific proteins etc⁶.

Enumerating specific cell types from whole blood is very useful for disease diagnostics⁷, one important example being counting of CD4 and CD8 T cells in HIV/AIDS diagnostics⁸. Microfluidic cell counters using Coulter method (based on changes in electrical impedance produced by nonconductive particles passing through a small aperture in an electrolyte) is fast and requires small volumes of sample. A biosensor has been developed based on a differential immunocapture technology to enumerate specific cells in 30 min using 10 μ l of blood.

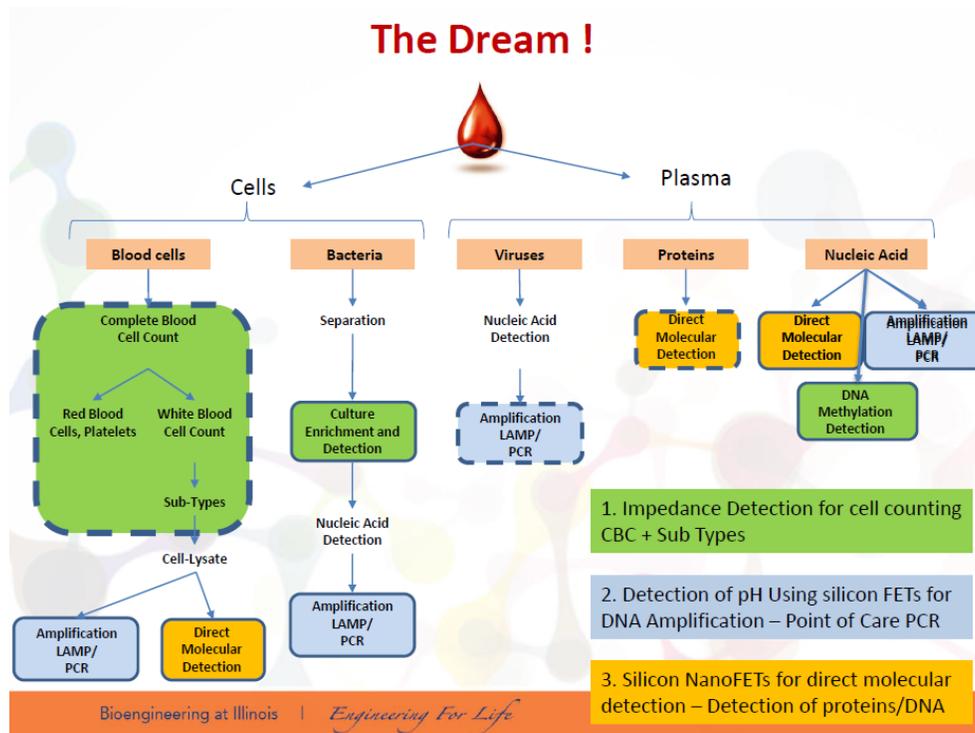


Fig. I. 4. A perspective on using microfluidic lab-on-the chip platforms for precision diagnostics⁶

Changes in electrical impedance produced by particles passing through a small aperture has also been applied for detection and analysis of different bio-molecules. The detection of target molecules is achieved by electrophoretically driving the molecules through nanometer-sized pores in thin membranes and simultaneously monitoring the modulation of nanopore ionic current. This method shows promise, e.g. for nanopore-based next generation DNA sequencing technology. Probability and state of different diseases, such as cancer can be assessed from patient’s genomic extracts. For example, the feasibility of cancer detection by analyzing DNA methylation on genomic extracts from bodily fluids using solid-state nanopores has been reported⁹.

Various dielectrics and metals have been used to make synthetic membranes for nanopores. However, the finite thickness (usually above 10 nm) of the fabricated membranes presents a limit on the spatial resolution of the measurements, making single nucleotide resolution difficult to achieve. 2D materials, such as graphene and MoS₂ that come form single layer with the same order of thickness as the nucleotide separation in a DNA strand, are attractive solution to this problem. Integration of stacked graphene and MoS₂ layers (Fig. I. 5) could enable identification of individual nucleobases in DNA^{6, 10}.

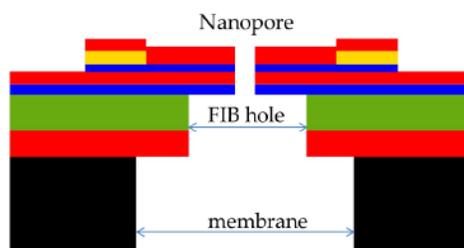


Fig. I. 5. MoS₂ nanoribbon on a graphene Al₂O₃ nanopore for DNA sensing

Interfacing Nanowires and Living Cells

Using one-dimensional (1D) semiconductors, such as nanowires (NW) allows creating mechanical and electrical interface with single cells and even monitor subcellular processes (Fig. 1. 6). The NW diameter can be as small as a few nm (comparable with the size of a single protein), while a typical length of the NW for biosensing applications is 1–100 μm , and thus the NW can span a single cell. They could be used as (quasi) non-invasive probes to contact or even puncture through the cell's membrane.

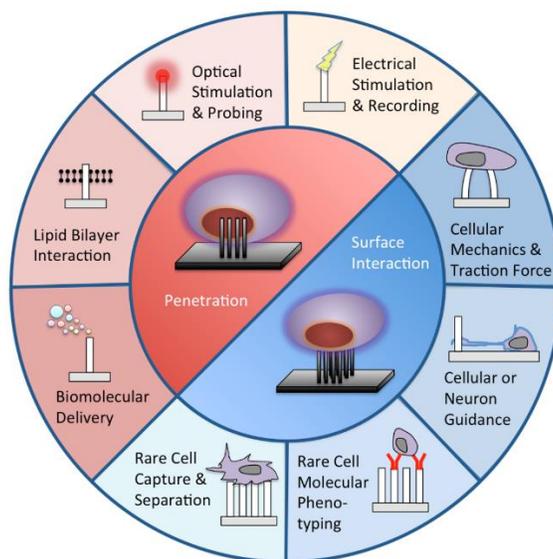


Fig. 1. 6. Interfacing inorganic nanowire arrays and living cells for a wide range of biological and biomedical applications^{11,12}.

Cell-penetrating inorganic nanowires can enable effective delivery of biomolecules, electrical and optical stimulation and recording of intracellular signals over a long period of time. Nanowire penetrations into cell through two mechanisms, namely through “impaling” as cells land onto a bed of nanowires, and through “adhesion-mediated” penetration, which occurs as cells spread on the substrate and generate adhesion force. Penetration is much more effective through the adhesion mechanism, with nanowire geometry and cell stiffness being critically important. Stiffer cells have higher penetration efficiency, but are more sensitive to nanowire geometry.

Non-penetrating interfacing of high-density nanowire arrays with cells has also been demonstrated. It is based on interactions between the nanostructured substrate and the micro/nanoscale features of cell surfaces. Such interactions enable efficient capture of rare cells including circulating tumor cells and trafficking leukocytes from complex bio-specimens. It also serves as a platform for probing cell traction force and neuronal guidance.

Nanowire substrate for rare tumor cell capture and quantitation in combination with laser scanning cytometry (LSC) technique allows for rapid, automated, high-content characterization of immobilized tumor cells.

Analysis of extremely low abundance cells such as trafficking leukocytes in cerebrospinal fluid (CSF) represents a significant challenge. An immune cell separation platform utilizing silicon nanowire surfaces was successfully employed for capture and analyses of trafficking leukocytes in CSF from the patients with Alzheimer's disease. Also, nanowire arrays for separation of rare cells demonstrated specific and efficient separation of CD4+ T lymphocytes from a highly heterogeneous mixture of immune cells.

While a variety of nanostructured surfaces demonstrated high efficiency immunocapture and separation of rare cells, the mechanism underlying such performance improvement remains unclear. Microvilli (cells 'nanohairs') can play a role in cells adhesion to nanowires. Live cell imaging of cell adhesion showed that when cells were captured on a nanowire substrate the cells quickly develop diverse subcellular surface features including filopodia and lamellipodia. Preliminary results indicate that the formation of filopodia on nanostructured substrates is correlated to the separation efficiency and a possible mechanism that led to enhanced efficiency of rare cell capture.

In summary, the physical interface between a living cell and a semiconductor material is complex and dynamics. How to design this interface at the nanometer scale confers new opportunities to control signal transfer between the two surfaces and the utilization of electrical, optical, or mechanical cues sent by semiconductor to modulate cellular function and activity.

Session 1 Roundtable Discussion Summary

The main focus of the roundtable discussion was future high-impact applications of hybrid cell-microelectronics systems. The roundtable participants shared a view that an aggressive application target is a wearable or implantable cellular control systems with artificial electronic cells and actual biological cells that will be used for predictive pathway cures in disease treatment. This could be realized in full scale in 10 years. Some essential components of the system could be realized within 5-7 years, for example highly stochastic biological - electronic computing systems that are fault tolerant and that solve problems that are highly inefficient on a deterministic computer.

A comment from the audience was that interfacing biological matter with electronics is a critical element of such hybrid systems. In current practice optical interface is overwhelmingly used which puts severe limitations on system's size, functionality and performance. Creating reliable direct electrical two-way communication channels should be a near-term research priority. While the panel in general agreed with this observation, some panelists also noted that optical and electrical communication channels can coexist concurrently in a bio-electronic system, analogously with the wireless vs. wired concurrency. Optical interface is 'wireless' and doesn't require direct proximity, however it has limitations on bandwidth, signal propagation, spatial resolution etc. Electrical connection allows for better sensitivity and locality, smaller energy consumption, compact system size etc. However, it requires a close proximity between biological and electrical components, which is often difficult to establish.

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- ¹ Rahul Sarpeshkar (Dartmouth Coll), “Cell-Microelectronics Systems for Computation”, ARO/NSF/SRC Technical Exchange Meeting on Cell-Semiconductor Interfaces and Hybrid Semiconductor-Biological Systems, Atlanta, GA, July 27 & 28, 2016
- ² J. J. Y. Teo, S. S. Woo, R. Sarpeshkar, “Synthetic Biology: A Unifying View and Review Using Analog Circuits”, *IEEE Trans. Biomed. Circ. Systems* 9 (2015) 453-474
- ³ Daniel Simon (Linköping U – Sweden), “Electronic Plants”, ARO/NSF/SRC Technical Exchange Meeting on Cell-Semiconductor Interfaces and Hybrid Semiconductor-Biological Systems, Atlanta, GA, July 27 & 28, 2016
- ⁴ A. Jonsson, Z. Song, D. Nilsson, B. A. Meyerson, D. T. Simon, B. Linderoth, M. Berggren, ‘Therapy using implanted organic bioelectronics’, *Science Advances* 1 (2015) e1500039
- ⁵ E. Stavrinidou, R. Gabrielsson., E. Gomez, X. Crispin, O. Nilsson, D. Simon, M. Berggren, ‘Electronic plants’, *Science Advances* 1 (2015) e1501136
- ⁶ Rashid Bashir (U Illinois), “Micro- and Nanoelectronic Devices for Medicine and Biology”, ARO/NSF/SRC Technical Exchange Meeting on Cell-Semiconductor Interfaces and Hybrid Semiconductor-Biological Systems, Atlanta, GA, July 27 & 28, 2016
- ⁷ U. Hassan, R. Bashir, “Electrical cell counting process characterization in a microfluidic impedance cytometer”, *Biomed Microdevices* 16 (2014) 697-704.
- ⁸ U. Hassan, N. N. Watkins, B. Reddy, G. Damhorst, R. Bashir, ‘Microfluidic differential immunocapture biochip for specific leukocyte counting’, *Nature Protocols* 11 (2016) 714–726
- ⁹ J. Shim, Y. Kim, G. I. Humphreys, A. M. Nardulli, F. Kosari, G. Vasmatzis, W. R. Taylor, D. A. Ahlquist, S. Myong, R. Bashir, “Nanopore-Based Assay for Detection of Methylation in Double-Stranded DNA Fragments”, *ACS NANO* 9 (2015) 290-300
- ¹⁰ S. Banerjee, J. Wilson, J. Shim, M. Shankla, E. A. Corbin, A. Aksimentiev, R. Bashir, “Slowing DNA Transport Using Graphene–DNA Interactions”, *Adv. Funct. Mater.* 25 (2015) 936-946
- ¹¹ Rong Fan (Yale U), “Interfacing Vertical Inorganic Nanowire Arrays with Living Cells for Cellular Function Analysis”, ARO/NSF/SRC Technical Exchange Meeting on Cell-Semiconductor Interfaces and Hybrid Semiconductor-Biological Systems, Atlanta, GA, July 27 & 28, 2016
- ¹² M. Kwak, L. Han, J. J. Chen, R. Fan, “Interfacing Inorganic Nanowire Arrays and Living Cells for Cellular Function Analysis”, *Small* 11 (2015) 5600-5610

Real-time bilateral interface between semiconductor platforms and biology (Session II)

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Cells are highly complex systems that often exhibit multi-physics responses under external stimulus. This session will focus on technologies for creating interfaces that can provide (a) single-cell (<5-10 μm /pixel) or sub-cell resolution (<1 μm /pixel), (b) real-time two-way communication (sensing and actuation by both cells and microelectronics), (c) multi-modality interfacing with cells, (d) compatibility with high throughput massively parallel operations, and (e) possibility of production at commercial quantities.

The nanometer-scale complementary metal-oxide semiconductor (CMOS) process is a potential candidate to realize cell-microelectronics interfaces. Electronics-based computations and signal processing, such as application of machine learning methods, may drastically relax the requirement on the physical interface and lead to further pixel miniaturization. In parallel, optoelectronics for interfacing with cells/tissues and various emerging technologies, such as nano-pores, silicon nanowires, and graphene biosensors, may greatly augment the CMOS.

The topics discussed at this session included multi-modal cell-semiconductor interface for hybrid systems, electrochemical camera chip for imaging multiple metabolites in biofilms, nano-Biophotonics for cellular and molecular detections, e- nanoconduits to electronically interface cells with materials, and three-dimensional silicon for subcellular interfaces.

Multi-Modality Cell-Semiconductor Interface for Hybrid Systems

Cells are often viewed as the smallest building blocks of life that constitute all living organisms. Typical cell sizes highly depend on the cell types, which range from 100 μm (human egg), to 8-10 μm (human red blood cells), and down to 1-2 μm (*E. coli* bacteria). Despite of their small sizes, cells are highly complex systems with numerous molecules operating concurrently in hundreds of pathways most of which are carefully regulated to maintain the cell phenotypes and proper cellular functionalities.

Understanding cells and leverage their built-in or synthetic functionalities will have a tremendous economic and scientific impacts. From the economic perspective, the cell biology and biotechnologies directly impact multiple large-volume and fast-growing markets in healthcare and pharmaceutical industries, such as cell-based assays dominated by drug discovery/testing (\$18.3B by 2020¹), stem-cell development (\$170.1B by 2020²), and regenerative medicine (\$67.5B by 2020³). On the scientific impacts, cells offer a wide variety of natural or synthetic functionalities (e.g., sensing, actuation, synthesis, computation, processing, and even energy harvesting) and can serve as an organic and highly versatile interface to the external environment (Fig. II. 1).

On the other hand, semiconductor technologies have evolved substantially over the past several decades and now become one of the most powerful man-made “inorganic” platforms for implementing sophisticated microscopic systems. Besides their unparalleled capabilities of computation, storage, mass-production, semiconductor technologies offer a wide variety of physical sensing and actuation functionalities, and their feature sizes (nm to μm) are notably suitable for cellular interfacing.

Thereby, recently there is an increasing interest in hybrid cell-semiconductor systems that can leverage both natural/synthetic biological processes and semiconductor technologies (Fig. II. 1). In such hybrid systems, it is expected that the major technical challenges and research opportunities lie in the “physical interfaces” between the “electronics/semiconductor layer” and the “biology layer.”

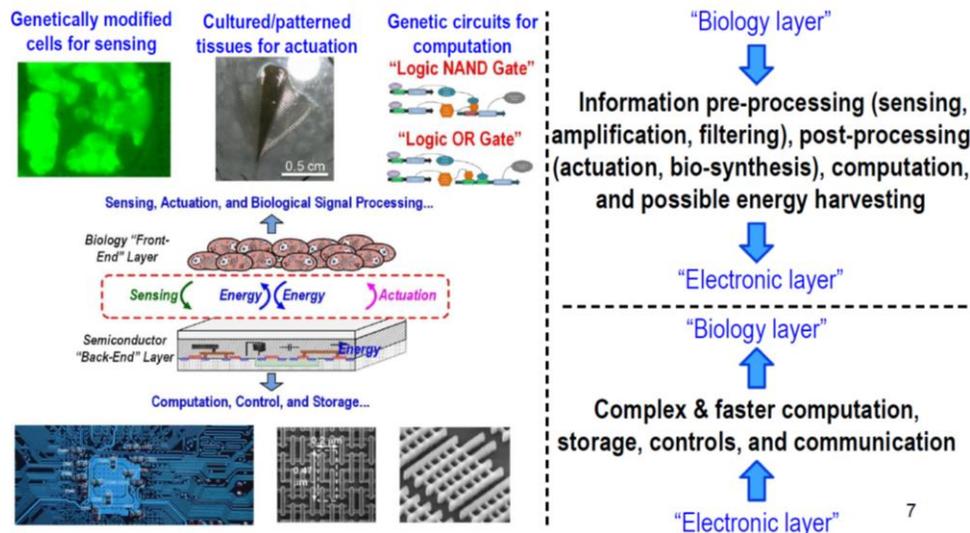


Fig. II. 1. Hybrid cell-semiconductor systems that leverage both natural and synthetic biological processes and semiconductor technologies.

Since cells are highly complex systems, it is becoming increasingly clear that multi-modal interfacing on cells is essential to capture the complex cell physiological changes, modulate cellular functionalities, enable holistic characterization and understanding on cells⁴ (Fig. II. 2). Typical cellular processes of interest include cellular potentials, cell-surface attachment, cell morphology, metabolism, molecular markers, while useful cellular actuations comprise electrical voltage/current, electrochemical reactions, thermal, mechanical, and optical processes. However, existing cellular sensors or actuators are mostly of single modality that cannot capture. On the other hand, semiconductor technologies, e.g., CMOS, support the cellular interfacing with all these modalities, besides their high spatiotemporal resolution and computational throughput. In addition, the potential large-volume and high-growth market related with cell biology also naturally match well with the economics of semiconductor industry that essentially relies on mass production of silicon chips and economic of scales. Therefore, it is envisioned that the semiconductor technologies (e.g., CMOS) can greatly benefit the cell biology and biotechnologies, while the latter also offer promising new markets that will potentially support the continuous growth of semiconductor industry in the “post-MOORE” era.

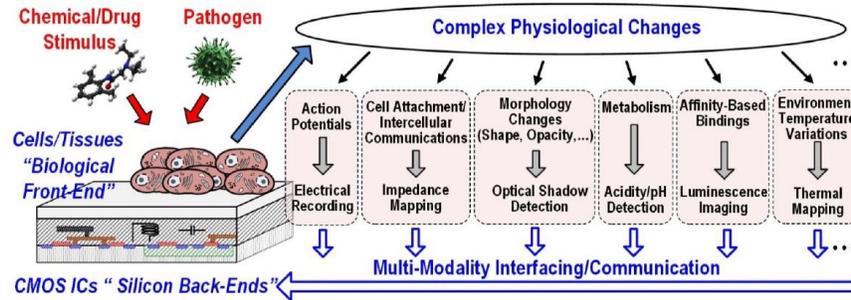


Fig. II. 2. Multi-modal interfacing with cells is essential to capture complex cellular physiological changes and achieve holistic cellular characterization.

In recent years, there have been substantial research investment on multi-modal cellular interface technologies using commercially available low-cost semiconductor technologies, such as CMOS. This area of research has been pioneered by Georgia Tech⁵⁻¹⁰. It has been shown that multi-modal cellular sensing arrays are achieved in CMOS processes (Fig. II. 3). The achieved array scaled is from hundreds of pixels/chip to over 10k pixels/chip, and the chip size is from 2mm×2mm to 7mm×7mm to support tissue level field-of-view (FoV). Each pixel supports multiple sensing modalities, including cellular potential, current, impedance, optical, and thermal detections, and each pixel can be individually configured and addressed. The pixel size ranges from 80µm×100µm to 16µm×16µm, approaching single-cell resolution for mammalian cells. The CMOS cellular sensing arrays also contain signal conditioning and pre-processing circuitry on-chip. Biocompatible packaging using medical epoxy and low-cost polydimethylsiloxane (PDMS) has been demonstrated to match standard multi-well plate for cell analysis. Biocompatibility has been verified using various mammalian cells and bacteria.

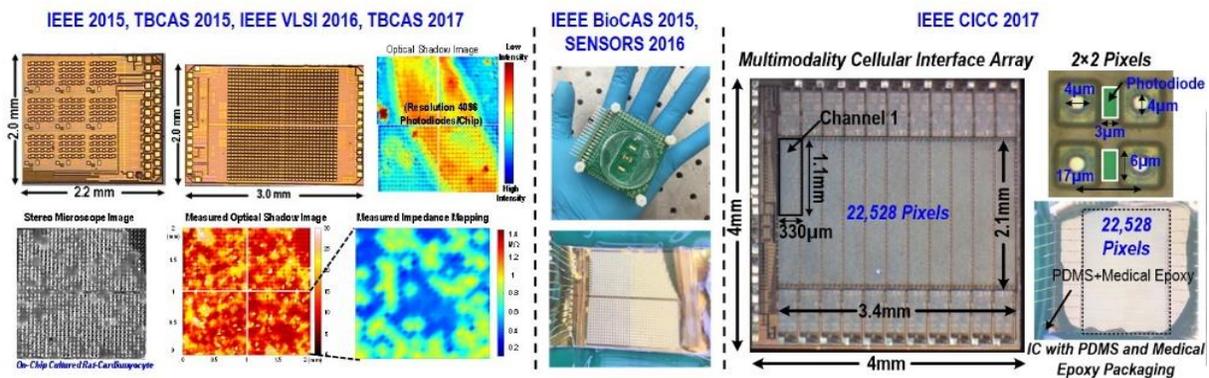


Fig. II. 3. Examples of CMOS multi-modal interfacing array chips for holistic cellular characterization, high throughput drug screening, and cell-based sensors.

The CMOS multi-modal cellular sensing arrays have shown their use in a wide variety of cell-based assays, including real-time bioluminescence imaging, real-time cell impedance and cell detachment/migration assay, joint recording of extracellular potential and opto-mechanical potentials. These assays use various cell types, such as human ovarian cancer cell aggregates (HeyA8-F8), mouse/human neuron cell clusters, rat cardiomyocyte clusters, and *E. coli* bacteria⁵⁻¹⁰. The joint recording of extracellular potential and opto-mechanical potentials is shown as an effective multi-parametric means for holistic cardiac cell characterizations and testing drug efficacy and cardiac toxicity.

It is believed that future hybrid cell-semiconductor systems will be a highly interdisciplinary field that require collaborative efforts and knowledge from areas such as electronics, device physics, micro-fabrication, biology, biochemistry, control theory, and signal process.

Electrochemical camera chip for imaging multiple metabolites in biofilms

Optical signals have been employed as the predominant means for interfacing the solid-state sensors and the cells for both *in vivo* imaging and molecular diagnostics. Light offers non-invasive interrogation, a diversity of developed and well established organic/inorganic chromophores, and also direct cell stimulation capability, e.g., optogenetics. However, optical interfacing has multiple disadvantages. Optical detection suffers from shot-noise and is mostly pronounced at high bandwidth. A single optical reporter only generate weak signal, which then solely relies on the electronics for signal amplification. In most cases, natural biophysical process do not generate light, and optical signals are only an indirect means for measurement and require labeling or genetic modification to add optical reporters. Moreover, most fluorescent measurements are restricted by photo-bleaching and cannot perform long-term real-time monitoring. Although multi-photon fluorescent excitations can alleviate this issue, it adds further complication on already complex optical measurement setups.

In contrast, using electrical interface will address many aforementioned disadvantages of optical interfacing. In particular, electrical measurements naturally captures various electrochemical processes that are essential in cellular and molecular characterizations. However, electrical transduction inherently requires close proximity and experience form-factor mismatch and signaling/system mismatch. The former is because of the geometrical and mechanical property difference between the cells and electronics, which can be reconciled by making electronics miniaturizing or flexible/conformal to the biology. The latter mismatch is due to the fact that biology using ions for their signaling, while electronics use electronics. Thus, bioelectrical interfaces should be either based on charge detections or electrochemical reactions that convert ions to electrons.

Two examples of bio-electrical interfaces are presented in this paper, i.e., a CMOS electrode array for electrochemical imaging of bio-films^{11, 12} and a packaged system that harvest energy from ATP to power integrated electronics¹³.

In the first example, the CMOS electrode array employs a large number of working electrodes that share one pair of counter electrode and reference electrode. The working electrodes also share multiple trans-impedance amplifiers (TIAs) that provide low termination impedance and current sensing capability. The number of working electrodes have been demonstrated to be 60 electrodes/chip to 1824 electrodes/chip. Thus, the CMOS electrode array chip achieves 3-electrode potentiostat with up-to 1824 sensing sites by working electrode multiplexing, which supports whole-chip scanning frame rate of 1 frame/second. Such CMOS electrode array thus can detect electrochemical reactions of deposited bio-films with high spatiotemporal resolution and function as an “electrochemical camera.”

Various electrochemical experiments have been demonstrated for detection of phenazines. Note that phenazines are a group of redox-active metabolites that are produced by *Pseudomonas aeruginosa* PA14, an opportunistic pathogen, which have drastic effects on community behavior in

colony biofilms and critical clinical relevance in understanding biofilms. The experiments show that phenazines can be detected with spatiotemporal resolution under different redox conditions.

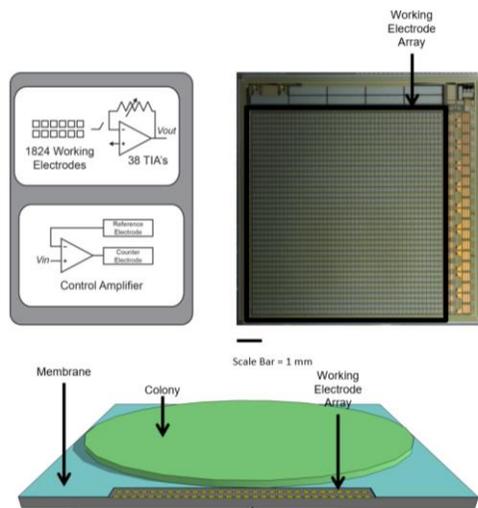


Fig. II. 4. Multi-modal interfacing with cells is essential to capture complex cellular physiological changes and achieve holistic cellular characterization.

In the second example, the packaged 3D system contains *cis* chamber (-) and *trans* chamber (+), separated by a delrin ring with 250 μ m aperture that contains bi-lipid layers embedded with sodium-potassium trans-membrane pump proteins. Mediated by ATPase, the hydrolysis of ATP to ADP releases free energy ($\text{ATP} + \text{H}_2\text{O} \rightarrow \text{ADP} + \text{P}_i$) with -30kJ/mol. This free energy drives the sodium-potassium pump and maintain the desired ionic gradient between the *cis* chamber (-) and *trans* chamber (+) to provide a desired bio-cell battery as a continuous electrical energy source. The total bio-cell battery achieves an averaged power of 163fW with an output open-circuit voltage of 80mV.

On the other hand, a typical low-power CMOS IC would require a supply voltage of at least $4-5 \text{ kT/q} \sim 125\text{mV}$ to ensure proper functionalities. Thus, a voltage boost-converter, e.g., a voltage doubler, is needed to up-scaled the supply voltage, so that the bio-cell battery can be useful for powering actual CMOS IC circuits. It is emphasized that such power converter circuit should be intrinsically low-power, low-voltage, and high-efficiency to handle the fW power from the ATP bio-cell battery. Overall, the efficiency of energy extraction from ATP bio-cell battery is 14.8%, in contrast to a calculated efficiency of 95% for similar ATP energy extraction in living systems, manifesting the superior efficiency of biological system when extracting and utilizing chemical energy.

Nano-Biophotonic Detections of Chemical Molecules, Proteins, Virus Particles, and Cells

Nano-photonics have been widely used for cellular and biomolecular sensing. Various nanostructures have been explored, including dielectric resonators, surface plasmons, optical waveguides, and Surface-enhanced Raman spectroscopy (SERS). This presentation will focus on the use of photonic crystals (PC) as an exemplar technology of nano-photonics for biosensing. Photonic crystals can be implemented as compact smartphone-based biosensors for environment/food monitoring or point-of-care patient monitoring. Moreover, photonic crystals can be utilized to achieve PC-enhanced fluorescence or PC-

enhanced microscopy, to potentially address applications such as disease diagnostics, life science research, and pharmaceutical screening.

A common surface photonic structure is “guided mode resonator filter” that functions as an optical resonator ($Q \sim 850$) for at least one wavelength. It is often constructed using periodically repeated structures that are coated with high refractive index material (SiN or TiO_2) between two materials with low refractive index (such as water, air, plastic, or SiO_2). It can be fabricated by nanoreplica molding that uses pre-patterned silicon master wafer as the mold to shape the liquid epoxy on a polyester sheet; the liquid epoxy is cured by UV light and then coated using high-refractive coating, such as TiO_2 . Such nanoreplica molding can achieve mass production at $\sim 0.5\text{m/min}$ rates as continuous rolls of polyester films. “Guided mode resonator filter” can also be fabricated on silicon substrates and glass wafers using post-processing lithography steps.

The “guided mode resonator filter” can be utilized for label-free biomolecular sensing and PC-enhanced fluorescence detections that enhance both the excitation EM field and the fluorescent emission profile with directional enhancement. A major application drive on next-generation photonic crystals research is to realize PC-enabled point-of-care diagnostics, which will ultimately replace slow and label-intensive laboratory tests by automated assays using low-cost sensors with access to “cloud” computing for data analytics. Recently, a PC special cradle is developed to convert a common cell phone camera into a spectrometer. Detection of HIV virus through affinity-based binding has been demonstrated^{14, 15}.

Next, several detailed application examples are presented to showcase the use of photonic crystal structures in various biosensing.

The first example is the PC-enhanced microscopy for label-free imaging of cell-surface interactions¹⁶, which utilizes the peak wavelength value (PWV) shift and peak intensity value (PIV) shift for surface-base label-free biosensing (Fig. II. 5). It has been shown that PC-enhanced microscopy can detect the surface attachments of various stem cell types and Au nano-rods¹⁶ with improved contrast over conventional bright-field imaging.

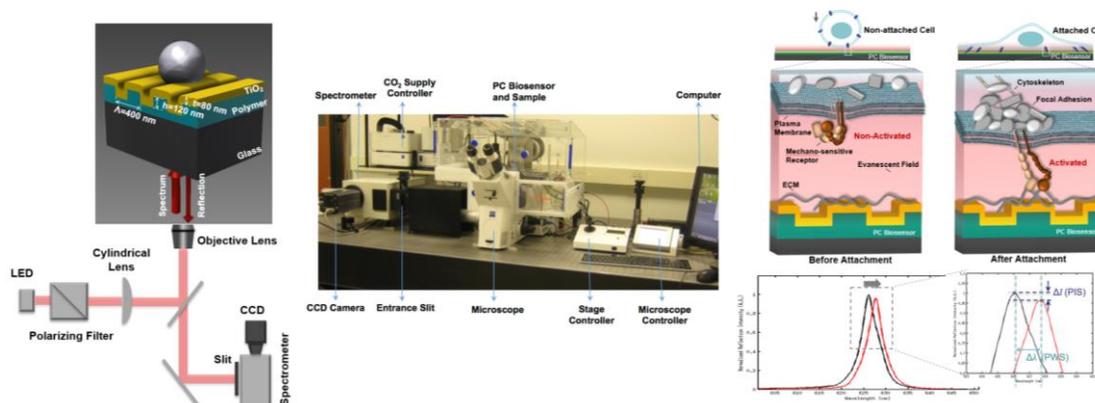


Fig. II. 5. PC Enhanced Microscopes for Cell-Surface Interaction Imaging.

The second example is external cavity laser biosensors for small molecule drug screening^{17, 18} (Fig. II. 6). The major challenge of drug screening is to efficiently search through libraries of chemical compounds to rule out most candidates and find those that have the desired interactions with the targets. The desired biosensor properties include high sensitivity, label-free detections, low-cost, and high

throughput to screen >100,000 compounds/day. Experimental results have shown that external cavity laser biosensors successfully detect dorzolamide within a mixture of 35 chemical compounds.

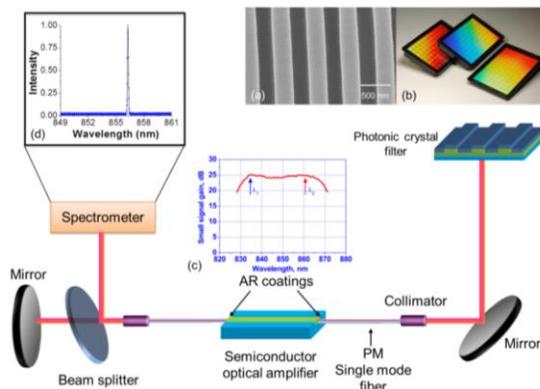


Fig. II. 6. External Cavity Laser Biosensor.

The third example is photonic crystal enhanced fluorescence for detection of cancer biomarker proteins¹⁹⁻²¹ (Fig. II. 7). The typical challenge of cancer biomarker detection is its extremely low concentration in blood circulation. It has been shown that PC-enhanced fluorescent imaging achieves better sensitivity than conventional unmodified glass-substrate of fluorescent imaging. Experimental results also achieve the lowest reported TNF- α detection concentration of 2.74pg/mL with replicate averaged SNR of 24.6. Similar enhanced fluorescent detections of various cancer biomarkers have been achieved by photonic crystal.

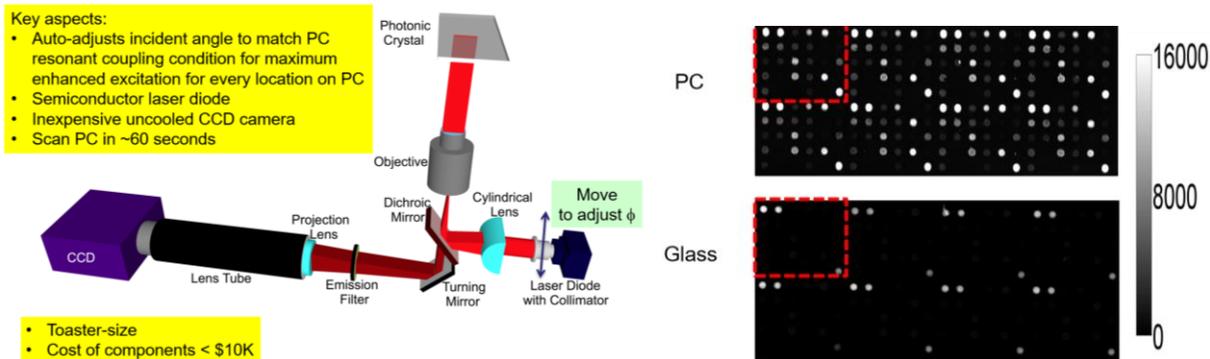


Fig. II. 7. PC Enhanced Fluorescent Imaging for Cancer Biomarker Detections and Comparison with Unmodified Glass Substrate.

e- Nanoconduits to Electronically Interface Cells with Materials

This presentation focuses on the fundamental scientific aspects of electron transfer between living cells and external electrodes, which is essential for creating cell-semiconductor hybrid systems. There have been recurrent discussions and debates on the major mechanisms of electron transfers across the cell membranes. It is believed that both cross-membrane electron nano-conduit and extracellular redox groups serve as media for electron transfer across the cell membranes.

This talk focuses on the fundamental science and application of cross-membrane electron nano-conduit.

It is reported that electron nano-conduits can be realized in synthetically engineered *E. coli* cells²²⁻²⁵. Such electron nano-conduits offer molecularly defined electronic control of electron flow across cell membrane. Moreover, it is shown that electron nano-conduits can potentially change the metabolism of bacteria by providing and modulating the electron channels across cell membranes²⁴.

Such study has also been extended to *S. oneidensis* MR-1 with a particular focus on whether electrodes can induce stress and increase protein turnover by the redox potentials²⁴. *S. oneidensis* was grown at potentiostatically poised electrodes at five redox potentials versus the standard hydrogen electrode (SHE) between -3 and $+797$ mVSHE. Subsequently, current production, coulombic efficiency, and transcription levels of marker genes for general stress and protein turnover were measured. Maximal current production was found at $+397$ mVSHE, and maximal coulombic efficiency was observed at $+197$ mVSHE. Both values decreased at more positive (oxidizing) potentials, that is, extracellular electron transfer of *S. oneidensis* is optimal at moderate electrode potentials. In contrast to previous findings, transcript measurements of a stress-marker gene indicate that extracellular electron transfer does not increase general stress in comparison with aerobic respiration. Although overall protein turnover is not related to electrode potential, increased expression of a protease suggests that protein degradation increases at oxidizing electrode potentials. Cyclic voltammetry reveals decreased activity of c-type cytochromes at the higher potentials, which indicates that oxidizing electrodes directly damage electron-transfer proteins at the electrode surface.

There are multiple interesting applications to utilize the electron nano-conduits (Fig. II. 8). For example, it is envisioned that plants can be genetically engineered to create electron nano-conduits on their cell membranes to form a massively deployed sensor network for complex molecules with kilo-meter coverage and center-meter spatial resolution. It is also demonstrated that deployable nanobioelectronic motes can be created using engineered bacteria to detect lactates.

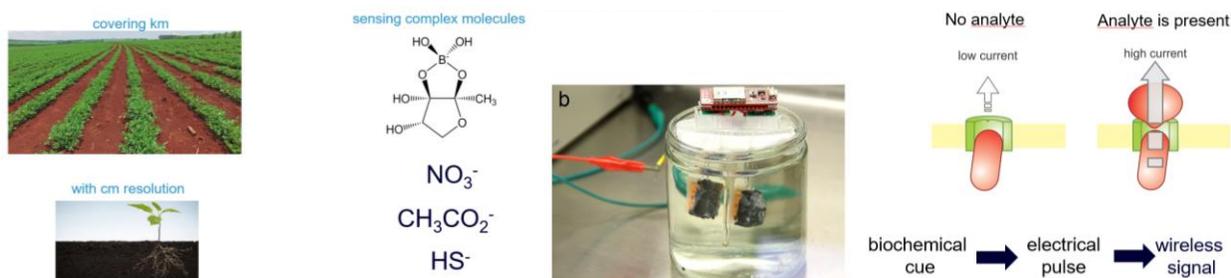


Fig. II. 8. Potential Applications of Electron Nano-Conduits in Massively Deployable Plant-Based Environment Sensor Network and miniaturized nanobioelectronic motes.

In summary, Electron nanoconduits offer a molecularly-defined route to efficiently move electrons in and out of living cells. Modulating flow of electron out of cells allows control of metabolism and deployable sensing of local environment. Extracellular electron transfer proteins use their 3D structures for recognition of specific materials.

Three-dimensional Silicon for Subcellular Interfaces

This presentation focuses on using 3-D silicon structures, their material, geometric, mechanical properties, and their interactions with cells to achieve sub-cellular interfaces with bioelectric and/or biomechanical modalities. Three technology examples are shown.

First, it is demonstrated that mesostructured silicon can be utilized to interface with lipid bilayers²⁶ (Fig. II. 9). Using mesoporous silica as the template, chemical vapor deposition system can implement the mesostructured silicon framework that show ordered nanowire array with amorphous properties. Mechanical tests show desired deformability of the silicon framework with its Young's modulus of 1.84 GPa in air and 0.41 GPa in liquid, which supports certain flexibility of the silicon framework when interacting with cells. In contrast, bulk silicon are very rigid with Young's modulus of 160~180 GPa.

It is shown that when the mesostructured silicon framework is placed in close proximity to phospholipid bilayer, a fast temperature variation can induce noticeable capacitive currents (up-to 100pA) in the phospholipid bilayer. Note that such temperature variation can be triggered by focused laser illumination. Moreover, the resulting capacitive currents can be used to trigger action potential of the cells. Thus, using mesostructured silicon framework and laser illumination, one can realize a remotely laser-controlled bioelectric interface for action potential induction. This structure is different from commonly used optogenetics, since there is no need for genetic modification of the cells.

It is shown that an average of 5.32 μ J threshold energy is needed to reliably elicit action potentials. The elicited action potential can track the optical excitation up-to 15Hz.

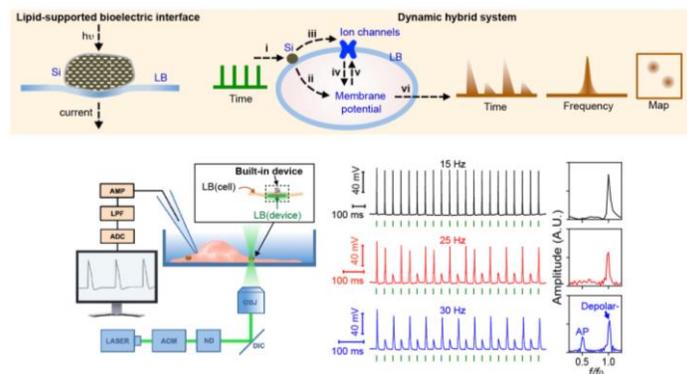


Fig. II. 9. A Silicon/Neuron Interface Using Mesostructured Silicon Framework.

Next, it is shown that silicon nanowires with kinked geometry can be used to probe the cytoskeleton and measure the intracellular mechanical properties²⁷ (Fig. II. 10). When silicon nanowires are co-cultured with cells, it is observed that the nanowires will cluster in the perinuclear region of the cells. If the nanowires are designed with kinked and asymmetric shape, and their geometry can be monitored using high-resolution microscope, their geometrical bending during the cell co-culturing can measure the intracellular force with both amplitude and location properties over time. In experiments, a maximum intracellular force of 116.9pN is measured and shows significant spatiotemporal evolution of intracellular forces.

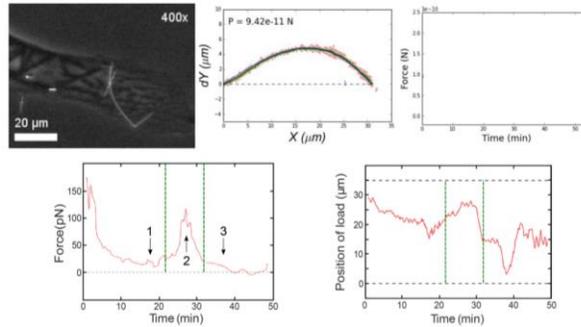


Fig. II. 10. Kinked Nanowires Allowed Intra-cellular Force Dynamics Study.

In the third example, 3D silicon nanowires are shown to measure the mechanical properties of extracellular matrix²⁸ (Fig. II. 11). The silicon nanowires can be designed to have anisotropy in their shapes, which then experience difference in insertion force and detachment force when probing extracellular matrix, yield useful information of cells and their environments.

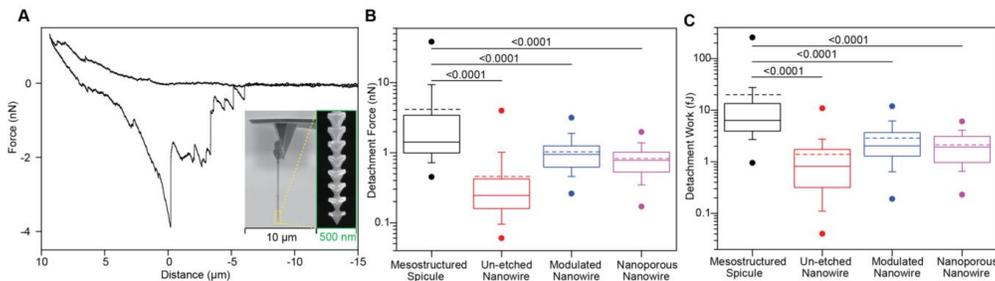


Fig. II. 11. Anisotropy in 3D Silicon Nanowires May Yield Unidirectional Biointerfaces .

In summary, 3D micromachined silicon or silicon nanostructures offer numerous new opportunities as biomaterials and cell-silicon interfaces with novel bioelectric and mechanical properties.

¹ Cell-Based Assay Market by Product (Cell Lines, Reagents, Kits, Instruments, Software, Services), Applications (Research, Drug Discovery, ADME Studies, Toxicology), End User (Pharmaceutical Companies, Academics, CRO), & Region -Global Forecast to 2020.

² Stem Cells Market Analysis By Product (Adult Stem Cells, Human Embryonic Cells, Pluripotent Stem Cell, Natural Rosette Cells), By Technology (Cell Acquisition, Cell Production, Cryopreservation, Expansion, Sub-Culture), By Application (Regenerative Medicine, Drug Discovery And Development) And Segment Forecasts To 2020

³ World Regenerative Medicines Market -Opportunities and Forecasts, 2013 -2020

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Handling, processing, and maintaining cellular samples on semiconductor surfaces (Session III)

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This session focus on the technologies to manipulate living cells and tissues and assemble them on a semiconductor surface. These technologies should potentially support high-throughput, scalability and low-cost implementation/operation. Also, among the challenges to implementation of cell-semiconductor systems is maintaining cell viability on silicon: Live cells will be integrated with CMOS technology to form a hybrid bio-semiconductor system, and keeping cells alive on silicon is a critical task for the cell-semiconductor systems. Fundamental and practical limits of enhancing the biocompatibility of semiconductor surface will be studied. In addition, we will also investigate the long-term reliability of the microelectronic interfaces in the biologically relevant environment, such as thin dielectrics with long-term robustness in the cell culture medium.

The topics discussed at this session included cell-matrix interface engineering, development of III-Nitride interfaces for sensing and cell studies, flexible and ultra-high density 3D heterogeneous packaging for biomedical applications, nano-biocomposites containing living cells, and 3D bio-printing and tissue engineering for creating new tissue constructs and functionalities.

Engineering the cell-matrix interface

It is well known that the material properties of the interface greatly impacts the cell-surface interactions. This talk reports recent progress on the understanding of microscale material properties of cells and how to develop and engineer “smart” materials that can mimic or even control the cell structures and cellular mechanics. The talk will focus on microfabricated culture systems and three dimensional hydrogels, how to engineer their material and mechanical properties, and how to utilize them as biomimetic platforms for screening and systems biology in tumor invasion¹⁻³.

Glioblastoma (GBM), as a common and probably the most malignant primary intracranial tumor, often exhibits tumor infiltration that causes the majority of mortality and subverts therapy. The annual incidence of GBM is 3-4 per 100,000, which is consistently rising. The median survival time is only 12-15 months even with brain resection, radiation therapy, and chemo-therapy. However, despite numerous research efforts, the median survival rate for GBM has not dramatically changed in almost 100 years.

It is thoroughly studied that the infiltration of GBM is profoundly impacted by the stiffness of its nearby tissues, and tumor cell invasion prefers stiff structures. However, tumor initiating cells (TIC) are found to be independent of the stiffness of nearby tissues. Experiments have shown that TICs can adhere, spread,

migrate, and proliferate equally well on soft and stiff ECMs. This is because TICs cannot generate contractile forces and thus cannot sense its nearby ECM mechanics. It is then found that by using RhoA, TICs can be engineered to generate contractile force, which re-introduces the “stop” signal associated with soft environment and also reduces GBM invasion through soft 3D matrices, achieving an expected 30% survival extension (Fig. III. 1)⁴. However, to fully exploit the therapeutic value of modulating cellular mechano-sensors, multiple technical challenges should be addresses.

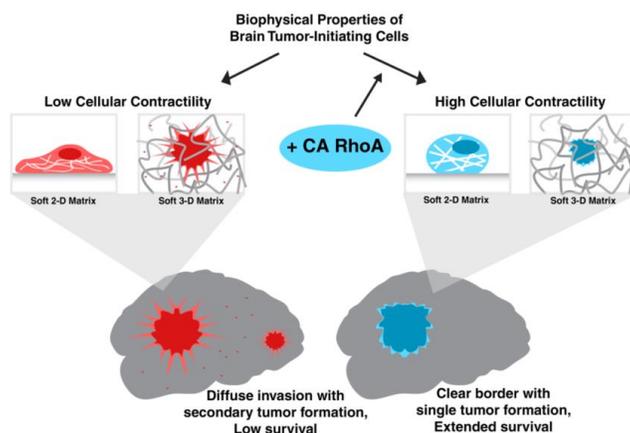


Fig. III. 1. Biophysical regulation of tumor progression and invasion

First, existing hydrogel platforms are poorly suited for cell screening to engineer their mechano-sensors. They are often labor intensive to fabricate, exhibit significant batch-to-batch variation, have extremely low-throughput, and may overlook the nonlinearity in data to misinterpret the stiffness of the material. It is shown that a cell screening platform can be achieved so that multiple experimental conditions of surface stiffness and ligand density can be tested on a single platform. It is achieved using hydrogel surface (e.g., employing hyaluronic acid as the polymer backbone) with spatially continuous gradients of stiffness and ligand density (e.g., fibronectin) in orthogonal directions. Thus, each position on the gel would encode for a different experimental condition. The polymer stiffness is modified by light, which is then verified by atomic force microscope⁵. Cancer cells are used to verify the principle, and one dual-gradient gel provides equivalent information content as 256+ serially fabricated gels, drastically improving the cell screening throughputs.

Next, it is realized that the bioactivity of extracellular matrix is derived from not only the mechanical properties but also the matrix architecture. Therefore, there is a need to promote 3D matrix architecture even on 2D surfaces. It is reported that using novel materials and surface chemistry, e.g., N-terminal specific method using 2-pyridinecarboxaldehyde (2PCA)⁶, one can substantially improve the 3D material on 2D surface, compared to conventional method such as using sulfo-SANPAH. The achieved advantages include uniform presentation of whole molecules to all the cells, minimal perturbation of bioactivity, and mobile chains to allow cells to remodel the proteins into fibers.

Development of Versatile III-Nitride Based Interfaces For Sensing and Cell Studies

It is generally believed that in order to optimize the biomolecular-inorganic interfaces, e.g., having good biocompatibility, there should a good matching between the valence band maximum/conduction band

minimum of the semiconductor surface and the HOMO (highest occupied molecular orbital)/LUMO (lowest unoccupied molecular orbital) of the biomolecules. On this note, III-nitride and III-oxide materials offer many advantages over silicon materials, since they provide chemical stability, wide and tunable bandgap, and a wide variety of surface chemistry or topology modification schemes.

It is experimentally shown that cell cultures achieve much improved cell density and adhesion on unmodified GaN surface and GaN surface modified with “IKVAV” peptide compared to modified or unmodified silicon surface⁶ (Fig. III. 2).

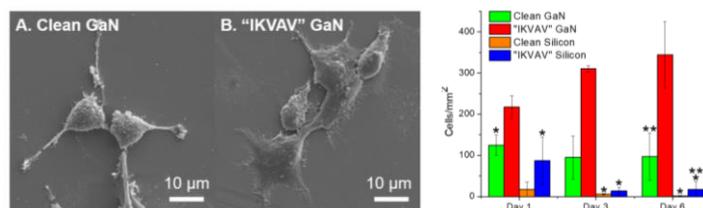


Fig. III. 2. P12 cell culture studies on GaN or Silicon surfaces.

In addition, it is known that nanoscale surface texture also influences cell response and cell cultures. It is then demonstrated that by incorporating Al to form $Al_xGa_{1-x}N$ materials, one can achieve tunability of bandgap, different impurity incorporation, different surface morphology, and potentially construct heterojunction devices. The study is further extended to *In Situ* functionalization of GaN with different polarities⁷ (Fig. III. 3). The chemical stability of the material is also proven using standard biological buffer solutions with or without incubators.

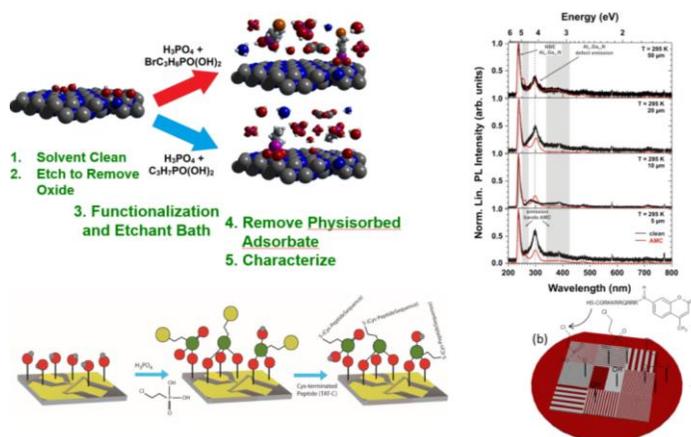


Fig. III. 3. *In Situ* Functionalization of GaN with Different Polarities.

Flexible and Ultra-high Density 3D Heterogeneous Packaging for Biomedical Applications

This talk seeks to leverage advanced 3D heterogeneous packaging technologies to augment solid-state sensors, e.g., CMOS sensors. In certain applications, direct cell culture on CMOS surface may lead to several technical challenges. Unsuccessful surface modification of CMOS electrodes may degrade their reliability in biological and aqueous environment and pose risks to both underlying electronics and cells under tests. Cleansing of the CMOS surface may not guarantee the elimination of contamination risks and may lead to electrode damage, which can significantly interfere with subsequent characterization

tests. Finally, native CMOS surface may prevent the addition of certain passivation layers that limit the biocompatibility and functionality of the biosensors.

An ideal cell-electronics interface are expected to offer the following capabilities. (1) Provide electrical/optical connections between sensing and CMOS electrodes. (2) Be disposable to circumvents contamination issue. (3) Provide a low cost option since CMOS biosensor can be reused. (4) Help to avoid potential damage to CMOS chip. (5) Support high pixel density for high resolution imaging while maintaining a large field-of-view. (6) Increase throughput and reduce cost.

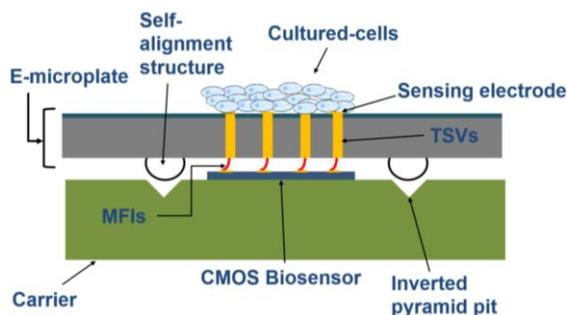


Fig. III. 4. 3D Heterogeneous packaging to improve interfaces between cells and CMOS biosensors.

It is demonstrated that advanced 3D packaging technologies can be leveraged to improve the interface between cells and CMOS biosensors (Fig. III. 4)^{8,9}. Glass, silicon or polymers can be used as the substrate materials of the interface to provide desired biocompatibility and support for surface chemistry. High-density TSVs (through-silicon vias) for electronic interconnections between the interfacing electrodes and the CMOS chip. MFIs (mechanical flexible interconnects) can provide stretchable and flexible interfacing with the CMOS chip, while inverted pyramid pits can be employed to allow self-assembly and –alignment of the interface with CMOS chip. The CMOS chip can be subsequently mounted on standard carriers.

Human CardioMyocytes (CMs) derived from embryonic stem cells (ESC) are cultured on 3D heterogeneous packaging. The autonomous beating spike periods are measured to be 5~7 s, which are confirmed by visual inspections through a microscope. Cell cultures are further performed to confirm the biocompatibility of the interface surface.

The future challenge is to further improve the spatial resolution down to single-cell resolution and provide both optical and electrical connections through the 3D heterogeneous interface.

Nano-Biocomposites Containing Living Cells

This talk focuses on a different area of hybrid cell-semiconductor systems, i.e., development of living biocomposites. These living biocomposites are expected to harness unique properties innate to biomolecules and living cells via 3D immobilization within matrices that preserve cellular behavior and accessibility to cells under ex-vivo conditions. They will also provide a unique and biocompatible interface between immobilized cells and the macro world.

One popular approach is to encapsulate living cells in silica matrices, which has attracted considerable attention in recent years. The silica matrices are mechanically stable, chemically and biologically inert,

easily processed at room temperature, retain water with negligible swelling, resist microbial attack, and can be tailored to provide desired porosity and other material and chemical properties. Various technical approaches exist to achieve compatible 3D silica matrices for cell immobilization, including cell directed assembly (CDA), cell directed integration (CDI), and the use of aqueous silicates.

It is demonstrated that using silica stabilized cellular communities, simultaneous fluorescent, electrochemical, and colorimetric detections can be achieved¹⁰ (Fig. III. 5). Multi-well cartridges can be prepared via laser ablated plastic laminates and lithographically defined ITO. Using multiplexing of sensing modalities and different cell lines, one can achieve complementary multi-modal sensing data to increase confidence in detection. It should be emphasized that using biocomposites, once sealed within device, living cells can remain viable and responsive when stored under ambient conditions for over 2 months, which is essential for future cell-based point-of-care or field-deployable sensor applications.

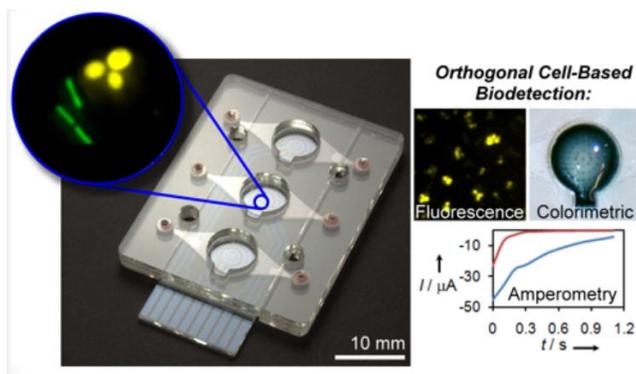


Fig. III. 5. Biocomposites and cartridge for simultaneous and orthogonal fluorescent, electrochemical, and colorimetric detections.

Moreover, biocomposite technologies allow co-culture and co-encapsulation of complete different cell types, even as eukaryote and prokaryote cells, in a silica thin film within an ITO/plastic laminate cartridge¹¹. Note that disparate cells typically do not remain viable together under standard culture. Various mammalian cells, such as *Danio rerio* (Zebra fish cells), bovine pulmonary artery endothelial cells, and primary rat cortical neuron, are shown to maintain their viability when being cultured in biocomposites.

Further studies has shown that confinement of cells in 3D matrices will substantially alter cellular behaviors, such as extension in viability, decreased (or no) replication, increased/decreased metabolic activity, confinement induced quorum sensing. The impacts on initial metabolic phases of *S. cerevisiae* cells upon encapsulation in a glycerol-silica matrix show that the cells experience two metabolic phases, i.e., the exponential phase with high metabolic activity and gene expression rates vs. the stationary phase with low metabolic activity induced by slow carbon starvation and split populations of 'mother' and robust quiescent 'daughter' cells (Fig. III. 6)¹². It is further shown that cells from exponential phase cannot quickly shift/adapt metabolism to respond to encapsulation stresses, while cells from stationary phase can maintain long-term viability (up-to 2 months) and consistent gene expressions. In addition, using *E. coli* bacteria, it is demonstrated that silica matrix composition can substantially influence bacteria bioactivity and viability in terms of membrane integrity, reproductive capacity, and metabolic activity.

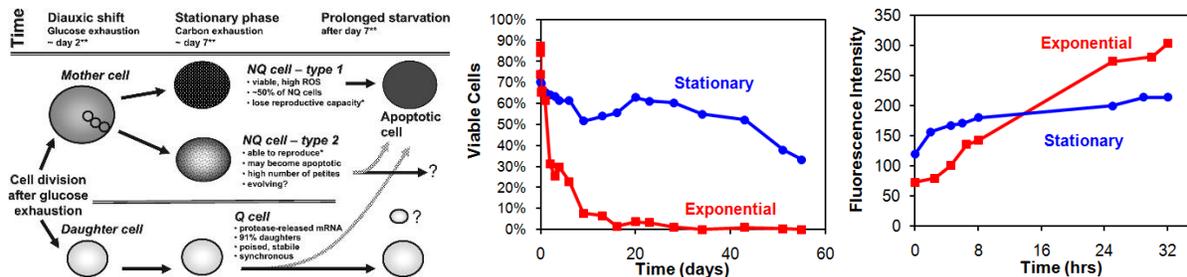


Fig. III. 6. Encapsulated cell metabolic phase effect on long-term cell viability and fluorescent viability testing.

In summary, living bio-nanocomposites have shown: (1) stabilization of a wide variety of cells for weeks to months under ambient conditions, (2) interfacing cells with nanomaterial functionalized microelectronic platforms allowing for multiple modes for monitoring cells, (3) co-stabilization of cellular communities, including disparate cell types for additional functionalities to hybrid systems, (4) significant impact of cell metabolic state on behavior, and (5) new and unexpected cell states and behaviors. However, our understanding of the bio/nano interface, in particular biocomposites, is in its infancy. Bio/Nano interface properties are key to tuning cellular behavior, which has more significant impact than bulk matrix chemistry or confinement alone. Moreover, the ability to induce, and perhaps control, a particular biological state may provide a powerful new tool for research in cancer, aging, cell-cell signaling, quorum sensing and development of novel bioelectronics.

3D Bio-printing and Tissue Engineering for Creating New Tissue Constructs and Functionalities

Biological tissues are probably among the most resilient, regenerative and functional materials. For example, human cardiac tissues generate and maintain persistent pulsatile beating at around 72 beats/min in their normal physiological condition, which is 38 million beats per year or 3 billion beats over the entire lifetime. The liver as an organ can regenerate after partial hepatectomy. In the context of biology-semiconductor hybrid systems, compared to other synthetic materials, the biological tissues offer many very unique and critical properties, including self-healing, adaptive architecture, self-renewable, natural stimuli responses, various sensing capability, and being actuatable by physical/chemical stimuli. Nature or engineered biological tissues can be used for disease models/drug discovery, regenerative medicine, cell-based biosensing, biorobotics, bioenergy-harvesting, artificial food, and biological science research (Fig. III. 7).

This talk focuses on leveraging 3D bio-printing and tissue engineering that can create new tissue constructs with novel or programmable new functionalities. In addition, advanced materials and micro/nanoscale technologies can be used to generate microenvironments to induce tissue formation. However, a major challenge lies in the fact that living tissues are usually constituted by smaller repeating units (hundreds of microns), containing different cell types, in a well-defined 3D architectures. New technologies on materials, micro/nano-fabrications, and tissue engineering are needed to mimic the complexity of native tissue architecture such as repeating tissue units with well-defined 3D architecture, and synergistic interactions of multiple cell type.

A few examples are presented to showcase the use of 3D bio-printing for new tissue constructs.

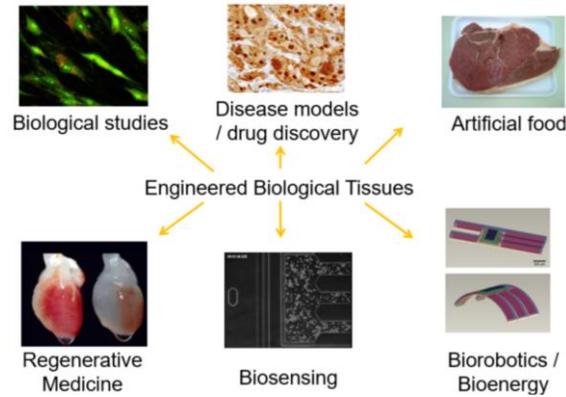


Fig. III. 7. Applications of Engineered Biological Tissues.

The first example is carbon nanotube embedded hydrogels for engineering cardiac constructs and bioactuators. The major challenges in cardiac tissue engineering are due to their viability, ultrastructural morphology, and most importantly the functionalities and electrical conduction of cardiac tissues. Thus, various electrically conductive materials should be used as the scaffold for cardiac tissue constructs. Carbon nanotubes (CNT) together with hydrogels are a promising candidate, since they can create conductive bridge in non-conductive porous materials and directly enhance protein expression and tissue culture. It is reported that various 3D actuators can be realized using single/multi-layer cardiac cells and CNT hydrogel hybrid structures for different motions when actuated by electrical stimuli.

The second example is bio-printing and organs-on-a-chip platforms. In the past, organs-on-a-chip systems mostly use 2D monolayer of animal cells with limited interactions between organoids. The research trend in this space is to utilize 3D organ constructs with primary human cells, iPS, etc. together with sophisticated microfluidics to construct interactive organoids, as a body-on-a-chip platform (Fig. III. 8). Research in this space has tremendous applications in drug screening and new drug discovery. Moreover, it is also envisioned that such a body-on-a-chip platform may replace animal testing to better predict human safety and efficacy of antidotes against biological and chemical weapons to secure military and civilian safety. It is shown that 3D bio-printing can be utilized to achieve 3D liver organoids, 3D cardiac tissues, vascularization, and even heterogeneous 3D constructs with vascularized tissues.

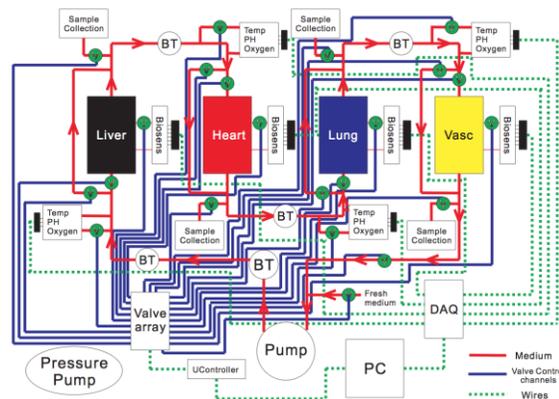


Fig. III. 8. Body-on-a-chip platform.

The last example is flexible smart wound dressing. The goal is to achieve real-time sensing, on-demand drug and tissue-derived chemical delivery to facilitate wound healing. The heterogeneous integration of biological and physiochemical sensing capabilities on polymeric substrates may lead to a paradigm shift in revolutionizing medical rehabilitation of chronic wounds. It is shown that stretchable Methacrylated Recombinant Elastin (MeTro) hydrogels can be used to realize designed patterning of cells, precise photo-masking, and flexible substrate for future smart wound dressing.

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Energy exchange between the cells/biological machineries and the microelectronic systems — Sustainable Energy Source (Session IV)

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In many energy-constrained applications, such as implantable devices, it is highly desirable to harvest energy directly or indirectly from the living cells/tissues and/or the surrounding biological environments to power the microelectronic systems. In the inverse case, energy flux that flows from microelectronics to the cells or the surrounding biological environments can impact biological function. For example, cellular respirations may be supported by electronic power. Also, heat generated by the semiconductor back-end could either be harmful or useful to the biological matter.

The topics discussed at this session included microbial electrochemical technologies as the power source for hybrid microelectronic systems and leveraging electrically conductive Pili — e-Pili (aka microbial nanowires) for electrode-microbe connections and sustainable electronic materials.

Microbial electrochemical technologies to power hybrid microelectronic systems

There is a rapidly increasing interest in using microbial fuel cells to generate electrochemical power. The microbial fuel cell (MFC) technology offers sustainable solutions for distributed power systems and energy positive wastewater treatment, but the generation of practically usable power from MFCs remains a major challenge for system scale up and application. This talk focuses on the fundamentals of microbial fuel cells, their state-of-the-art output power capability, current limitations, and future research directions.

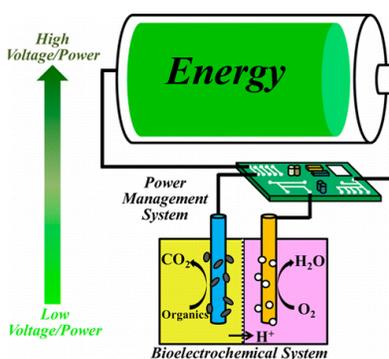


Fig. IV. 1. Basic structure of a bacterial fuel cell or microbial fuel cell.

A microbial fuel cell (MFC) is a device that converts chemical energy to electrical energy by the action of microorganisms, i.e., bacteria¹ (Fig. IV. 1). These electrochemical cells are constructed using either a bioanode and/or a biocathode. Most MFCs contain a membrane to separate the compartments of the anode (where oxidation takes place) and the cathode (where reduction takes place). The electrons

produced during oxidation are transferred directly to an electrode or, to a redox mediator species. The electron flux is moved to the cathode. The charge balance of the system is compensated by ionic movement inside the cell, usually across an ionic membrane. Most MFCs use an organic electron donor that is oxidised to produce CO₂, protons and electrons. Other electron donors have been reported, such as sulphur compounds or hydrogen. The cathode reaction uses a variety of electron acceptors that includes the reduction of oxygen as the most studied process. However, other electron acceptors have been studied, including metal recovery by reduction, water to hydrogen, nitrate reduction and sulfate reduction.

In practice, MFCs can generate electrical energy at the power density levels from sub- $\mu\text{W}/\text{cm}^2$ to almost $100 \mu\text{W}/\text{cm}^2$, depending on the reactor chamber constructs, bacteria communities, load optimization, and various other aspects²⁻⁵. The major challenges in wide deployment of MFCs include its low output power, low open-circuit voltage, and instability under changing environment conditions. To optimize the MFC operations, one should engineer the electrical power management system, the organic fuel supplies, and the microbes that often serve as the anode catalyst.

Advanced electrical power management systems are needed, since the theoretical open circuit voltage of a single acetate/O₂ cell is 1.1 V, while a typical voltage for single acetate/O₂ cell is 0.8 V. This low supply voltage can support various low-power operations such as wireless sensor nodes, but is insufficient for medium-/high-performance operations, which requires power management circuits. Moreover, maximum power point (MPP) tracking is needed to maximize the power harvesting.

For the organic materials, i.e., fuels or nutrition, for the MFC, it is shown that even <1 g sodium acetate can power a $10 \mu\text{W}$ device for 1 year⁶, showing the superior efficiency of MFCs.

Regarding the anode catalysts choices, homogeneous communities such as *G. sulfurreducens* may achieve high efficiency, while mixed microbe communities can be self-sustaining and environmentally robust in practice. Moreover, it is shown that using synthetic biology techniques, engineered bacteria can substantially improve the performance of anode catalysts in MFCs with up-to 14 times improvement in power generation⁷. In addition, synthetic biology also adds new functionalities to MFCs, such as achieving arsenic-specific biosensor in environmental monitoring⁸.

However, the grand challenge in MFC technologies remains as its low power density. Interdisciplinary research efforts are needed to increase MFC power density from $\mu\text{W}/\text{cm}^2$ to $\mu\text{W}/\text{mm}^2$ and beyond.

Making Electrode-Microbe Connections and Sustainable Electronic Materials with Electrically Conductive Pili — e-Pili (aka Microbial Nanowires)

Through natural evolution, Geobacters employ e-Pili to sustain their living where there is no O₂. This talk focuses on different advantages the potential applications of Geobacter e-Pili.

E-Pili can transfer electrons from Geobacters to nearby Fe (III) oxides, and adaptive evolutions select Geobacters whose mutations in regulatory systems result in increased expression of Pili. Multiple experimental evidence have shown that Geobacter Pili are indeed the conduits for long-range electron transport to Fe(III)⁹, since Pilin genes specifically expressed during growth on Fe(III) oxide; knocking out pili gene eliminates Fe(III) oxide reduction; and Pili are electrically conductive across their width.

Moreover, it is found that the conductivity of individual *Geobacter sulfurreducens* Pili at pH 7 is around 50 mS/cm, which is more than sufficient to account for observed rates of extracellular electron transfer to Fe(III), other cells, or electrodes. The conductive Pili of *Geobacter* can facilitate formation of thick electrically conductive biofilms with conductivities that rival those of synthetic conducting polymers¹⁰.

Further, adaptive evolution for higher current production can lead to greater Pilin production and higher conductance¹¹. Electrode-selected strain KN400 can achieve 10-fold higher power output per cell by expressing more pili and fewer outer surface cytochromes, resulting in higher biofilm conductance. Following studies also show that e-Pili model for biofilm conductivity as a predictive synthetic strategy to enhance biofilm conductivity. For example, engineered strain CL-1 can produce highly cohesive biofilms that are more conductive and generate higher power densities.

Major applications of these engineered and enhanced Pili expressions are microbial fuel cells. For example, microbial fuel cells can be used to power naval electronics by directly harvesting electricity from sediments¹². Microbial electrosynthesis is another application that can produce fuels and other organic chemicals from CO₂, water, and electricity. In addition, using microbes, one can perform bioremediations of contaminated soil, including uranium contamination and chlorinated solvent contamination (Fig. IV. 2). Thus, the ability of microorganisms to sense many environmental components as well as interact with electrodes offers the potential to devise various novel, sensitive sensors for unique environmental signals.

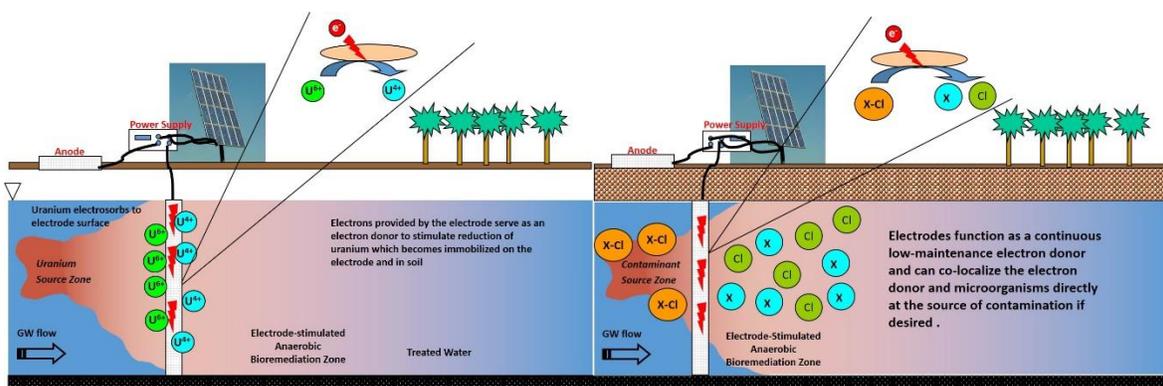


Fig. IV. 2. Feeding Microbes Electrons for Bioremediation of Uranium Contamination and Chlorinated Solvent Contamination.

In parallel, a mutation in PilA, the structural pilin protein, leads to the e-pili structure that are found only in *Geobacter* and their closely related microbes. It is shown that e-Pili with new properties can be produced by genetically modifying the PilA gene sequence, providing a new degree of freedom of e-pili designs. It is demonstrated that tryptophan can enhance short-range electron transport and thereby the pilus conductivity. The engineered biowires achieve 5000-fold higher conductivity and half the diameter of wild type e-pili¹³. Such a higher conductivity is verified at different pH levels (pH=2, 7, and 10.5). compared to other organic nanowires, synthetic e-Pili achieves conductivity between 2×10^2 - 10^3 s/cm, showing the best reported conductivity and even close to carbon nanotubes (Fig. IV. 3).

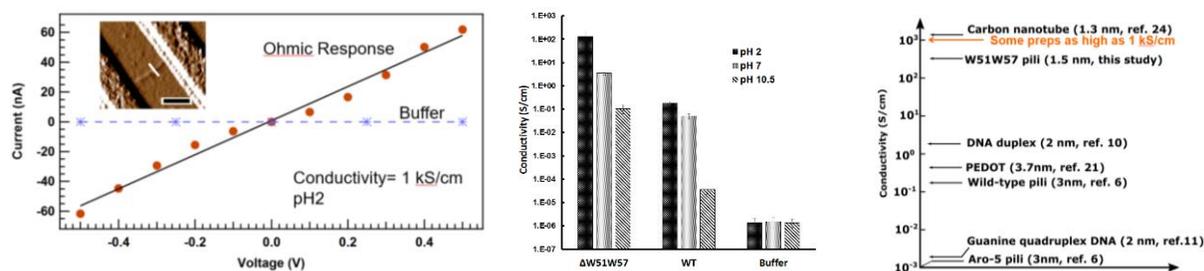


Fig. IV. 3. Measured conductivity of synthetically engineered e-Pili, conductivity at different pH values, and conductivity comparison with other organic nanowires.

Future research in this space will focus on further increasing the biowire conductivity and also expanding their functionalities. The biowire conductivity can be improved by further empirical manipulations of PiliA sequence, determining structures in a more knowledge-based manner, and continue prospecting in the vast reservoir of PiliA sequences found in nature. Functionalization of the biowire may involve metal binding sites, electron transport proteins to make electrical connections¹⁴, and conjugation with abiotic polymers. In conclusion, e-Pili as biowires may offer sustainable and radical solution for future electronic connectivity applications.

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Brainstorming: Synthetic biology for hybrid cell-microelectronics systems (Session V)

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Hee Cheol Cho / Emory University
Jake Beal / Raytheon BBN Technologies
Adam Meyer / MIT
Andrew Hessel / Autodesk

This session is composed a mixture of various topics that utilize synthetic biology for hybrid cell-microelectronics systems.

Biological Computing

Synthetic biology is an interdisciplinary branch of biology and engineering. It combines various disciplines, such as biotechnology, genetic engineering, molecular biology, molecular engineering, systems biology, biophysics, electrical engineering, computer engineering, control engineering and evolutionary biology, to build artificial biological systems for research, engineering, and medical applications. A major challenge in synthetic biology is to develop methodologies and tools to efficiently program DNAs and leverage these genetic components in living cells for computation and memory. In the context of cell-based sensors, such molecular signal processing and memory may achieve in-vivo conditioning of the detected information, making decisions in the biological frontend, and generate responses to ease down-stream processing (Fig. V. 1 and Fig. V. 2).

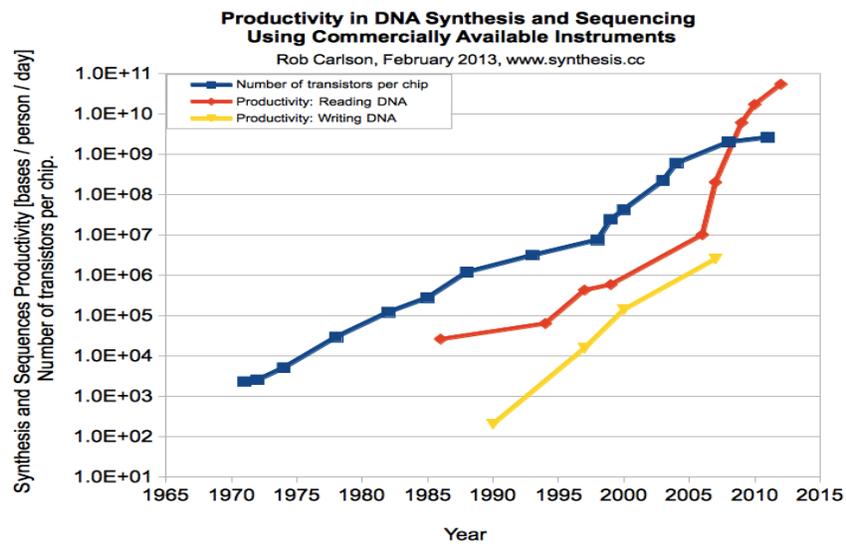


Fig. V. 1. Productivity in DNA Synthesis and Sequencing Using Commercially Available Instruments versus CMOS Integration Level.

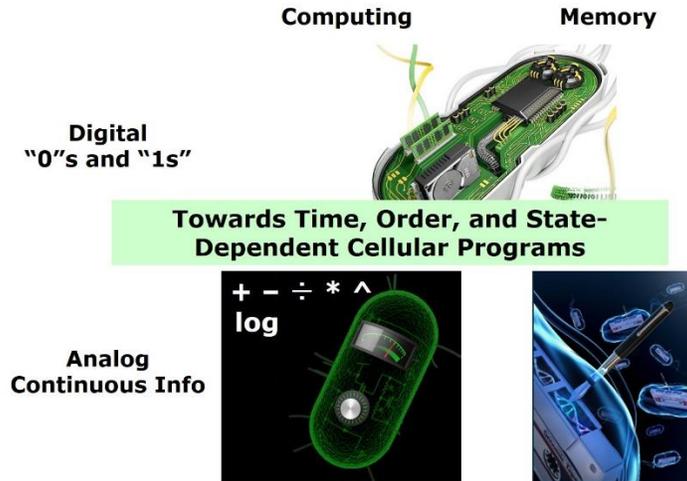


Fig. V. 2. Digital and analog computing and memory in living cells.

It is demonstrated that DNAs can be utilized as *in vivo* computation and memory in cells in both analog and digital paradigms¹. Moreover, one can achieve extensible, modular, “non-volatile” genetic memory, which offer digital behavior by encoding bits into DNA orientation, stability analogous to static memory, extensibility by >100 known invertases, and modularity designs with independent building blocks.

A wide variety of logic gates can be achieved using DNAs in living cells¹ (Fig. V. 3). Besides combinational logics, DNA circuits can also achieve sequential logics², for example counting and detecting unique event sequences. The DNA circuits can potentially realize scalable control of $>2^N$ gene expression states with N inputs.

By cooperating the combinational and sequential logics, it is further shown that one can encode DNA-based state machines in living cells, e.g., a 2-Input, 5-State finite-state machine (FSM) and a 3-Input, 16-Output FSM with large serine recombinases³. In a scalable DNA-based computation system, it is envisioned that the recombinase-based FSM can achieve 3.9^N states and combinatorial computing can realize 2^N states with N inputs (Fig. V. 4).

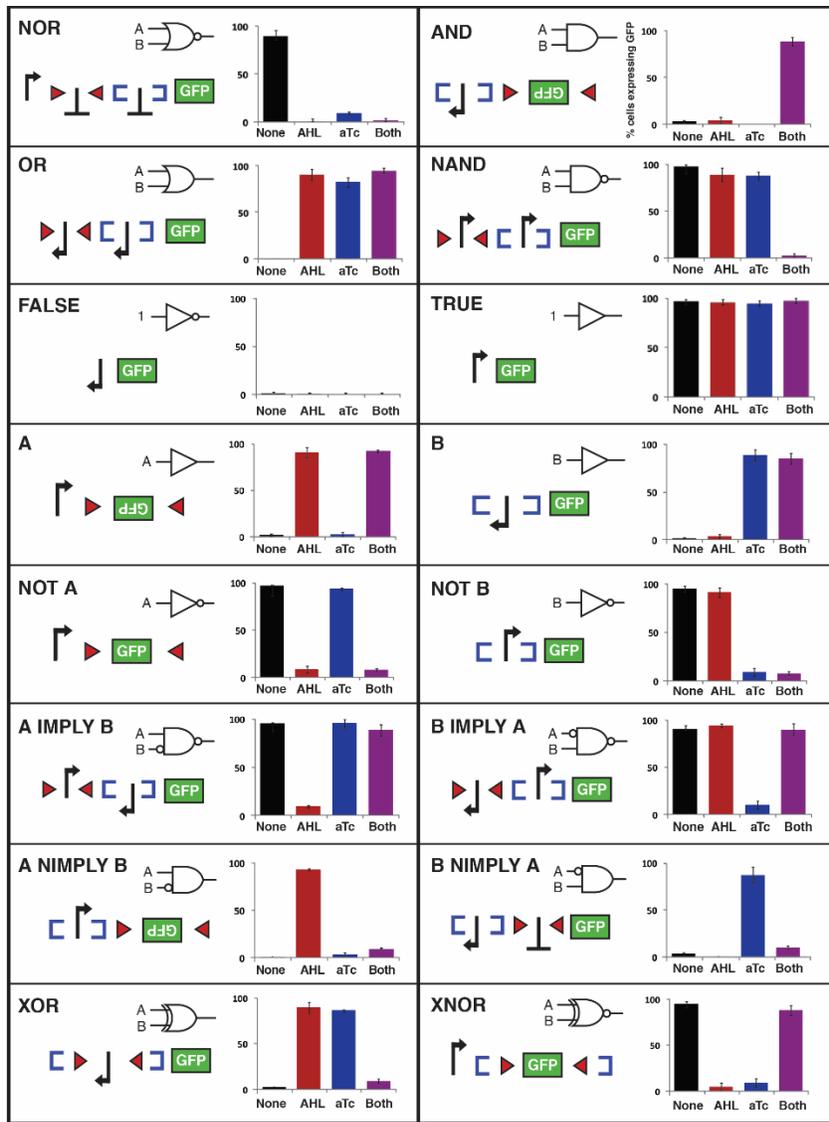


Fig. V. 3. DNA based logic gates.

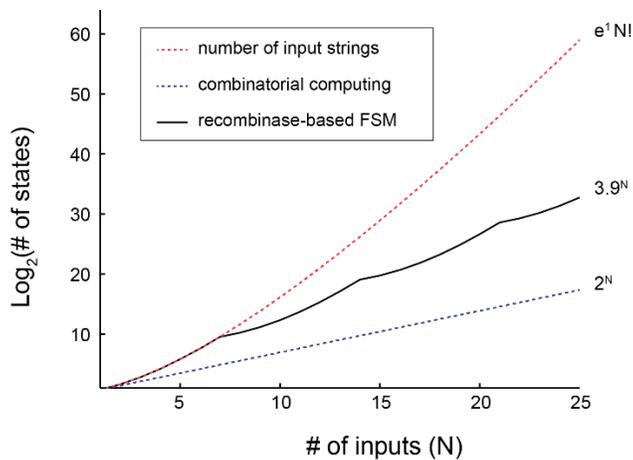


Fig. V. 4. Scalability of DNA based FSM and combinatorial computing.

Pacing the heart with genes and synthetic pacemaker cells

The heart requires a steady rhythm and beating rate to fulfill its physiological role as the pump for the blood circulation. An excessively rapid heart rate (tachycardia) results in insufficient time for the mechanical ventricular emptying and filling. This leads to a decreased cardiac output, lung congestion, and even a collapsed blood circulation. An equally morbid chain of events ensues if the heart beats too slowly (bradycardia). Serious disturbances of cardiac rhythm, i.e., arrhythmias, afflict more than 3 million Americans and account for over 479,000 deaths annually. In 2001, \$2.7 billion (\$6634 per discharge) was paid to Medicare beneficiaries for cardiac arrhythmia-related diseases.

Although the cardiac arrhythmia is a serious threat to the public health, current treatments however remain either inadequate or largely palliative with substantial limitations.

Anti-arrhythmic drugs are sometimes effective, but their utility is limited by their propensity to create new arrhythmias. Ablating cardiac tissues can cure simple electrical conduction errors, but it is less effective in treating complex and common arrhythmias, such as atrial fibrillation or ventricular tachycardia. Electronic implantable devices, e.g., pacemakers or defibrillators, can sustain heart rate or suppress tachycardia by delivering electrical shocks. Unfortunately, these electronics-only devices face a number of fundamental issues, including the inability to real-time detect the patients' physiological conditions and adjust the electronic pacing rate dynamically. Although several electronic pacemakers attempt to interpolate the patients' physiological needs by the cardiac output and blood velocity, obtaining the full physiological conditions by electronics-only devices still remains elusive.

On the other hand, recently there is an increasing interest in treating arrhythmia by a "biological pacemaker" approach using cell-based therapies^{5, 6}. It utilizes cell-/gene-delivery to convert a normally quiescent region of myocardium to "pacemaker cells", which can beat autonomously and further stimulate the entire heart. Compared with the electronics-only pacemakers, the "biological pacemaker" has the unique advantage of synthesizing natural cardiac beatings. Moreover, these biological pacemaker cells offer built-in molecular receptors and cellular machineries, which allow them to spontaneously adjust their beating rate in response to the real-time patient physical activities, neurotransmitter release, hormone level changes, and pharmacologic stimuli.

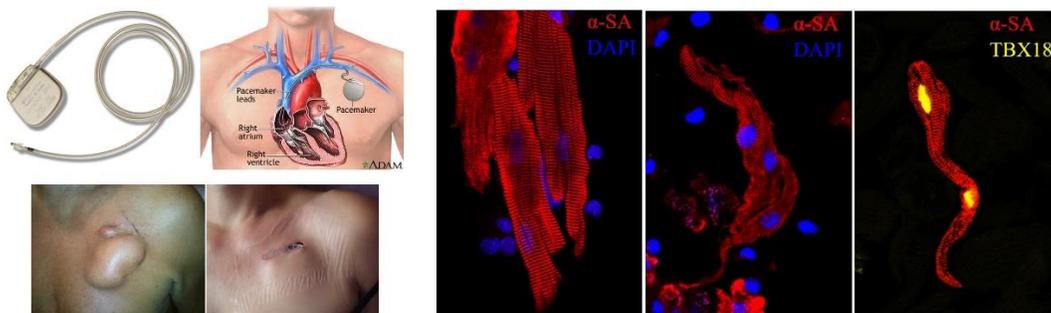


Fig. V. 5. Conventional electronic pacemaker and genetically engineered "pacemaker cells" for biological pacemaking.

The engineered "pacemaker cells" can be achieved by genetic programming ventricular myocytes through the Tbx18 gene to resemble the embryonic development of the SA nodes. The engineered ventricular myocytes show similar morphology and desired automaticity (action potential generation) to

natural SA node cells⁶. The “pacemaker cells” are then implanted to a porcine model with complete heart block. It shows that the “pacemaker cells” are able to create reliable biological pacing and restoring the functionality of the porcine heart rhythmic beating⁷ (Fig. V. 6).

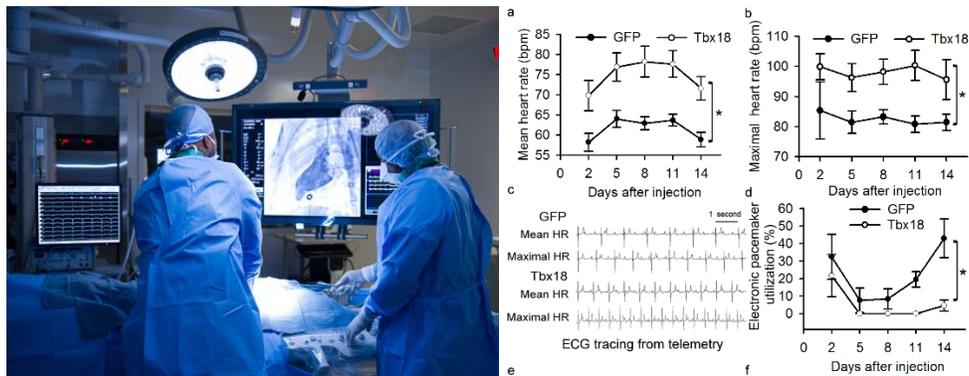


Fig. V. 6. Verification of the “pacemaker cells” and their achieved biological pacing⁷.

Hybrid assays for precision design of genetic regulatory networks

This presentation presents the concerns on the limited characterization capabilities and result variations in synthetic biology research and delineate future research direction of hybrid assays that may address these concerns (Fig. V. 7).

Despite the tremendous research investment, synthetic biology is still in its infancy. Engineering organisms and creating new organisms still lacks repeatability and result reliability. It is believed that the future vision should be “WYSIWYG — What You See is What You Get,” so that the engineered organisms can be produced and even mass manufactured faithfully base on their designs.

Such WYSIWYG vision necessitates precision characterization capabilities and predictive models. This is crucial since biological systems exhibit large variation in behaviors due to many classes of cause, including: (1) Inherent process stochasticity in transcription, translation, replication of genetic information, (2) Cell-to-cell differences in terms of their size, cycle state, health, mutations, location, (3) Protocol stochasticity due to transfection variation, insertion site, etc, and even (4) Protocol execution issues, such as reagent variation, contamination, instrument drift. These variations also exhibit distinct characteristics. The inherent genetic level randomness is often largely uncorrelated effects on individual system elements. The cell-to-cell variations can be highly correlated effects on individual system elements. The protocol stochasticity may be predictable distributions, affected by choice of protocol parameters, while the protocol execution issues are mostly unpredictable and must be detected and appropriately compensated.

Unfortunately, such undesired variations are almost ubiquitous in biology, and quantifying all the variation components requires per-cell measurements of large populations of cells. However, it is believed that the conventional high-throughput flow cytometry is not enough due to various limitations. First, flow cytometry are essentially disruptive measurements that lose the spatial context of tissues and only show one time point. There is no subcellular imaging. It is also difficult to distinguish very large or

very small cells. In addition, the popularly employed fluorescence is only an indirect and lagged signal indicator of the cells and are limited by tagging techniques and limited number of probes.

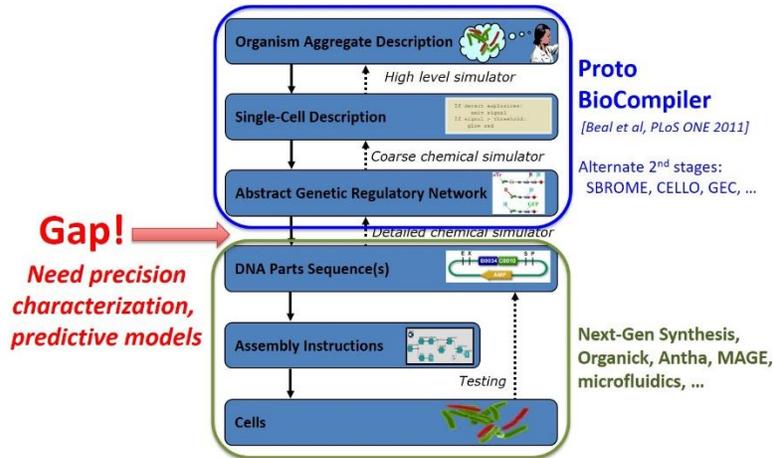


Fig. V. 7. Verification of the “pacemaker cells” and their achieved biological pacing.

It is believed that an “ideal platform” should support a large-scale continuous assays (Fig. V. 8). It should handle 100,000 cells at <math><20\mu\text{ms}</math> spatial resolution and support both high and low confluence. It should provide a field-of-view (FoV) of $\sim 10\text{mm}/\text{side}$ CMOS chip and can be calibrated by beads. Ultimately, it should support a target $5\mu\text{m}$ resolution as a 4 megapixel sensor array, which can support multi-modal sensing and can screen the entire FoV for all the cells within only a few minutes.

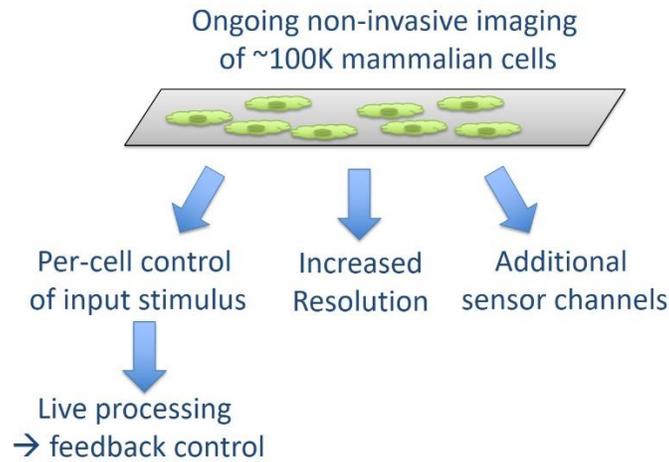


Fig. V. 8. Vision: large-scale continuous assay.

Directed evolution of an array of orthogonal biosensors

It is well understood that living cells can naturally sense, integrate and respond to various different stimuli, a combination of stimuli, or a sequence of stimuli (Fig. V. 9). Moreover, cells can also inherently convert stimuli and genetic outputs to different physically signals that can be measured by electronics (Fig. V. 10).

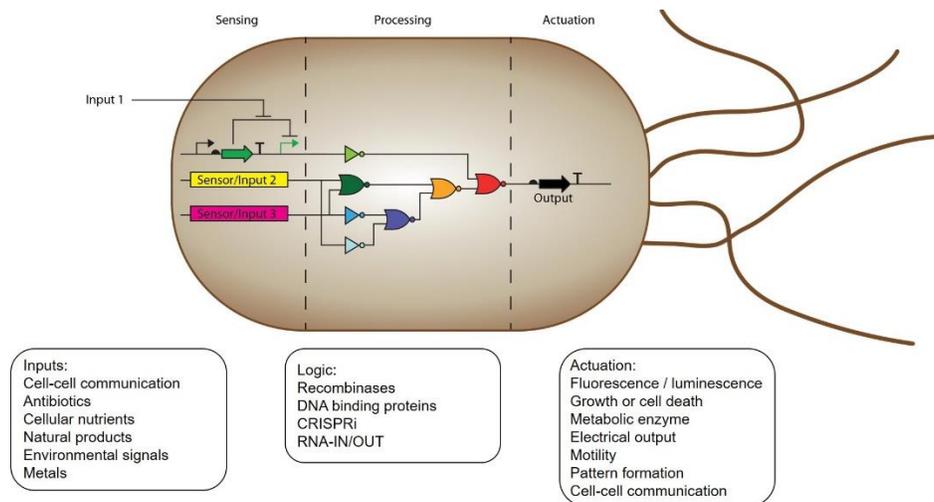


Fig. V. 9. Cells can naturally sense, integrate, and respond to stimuli

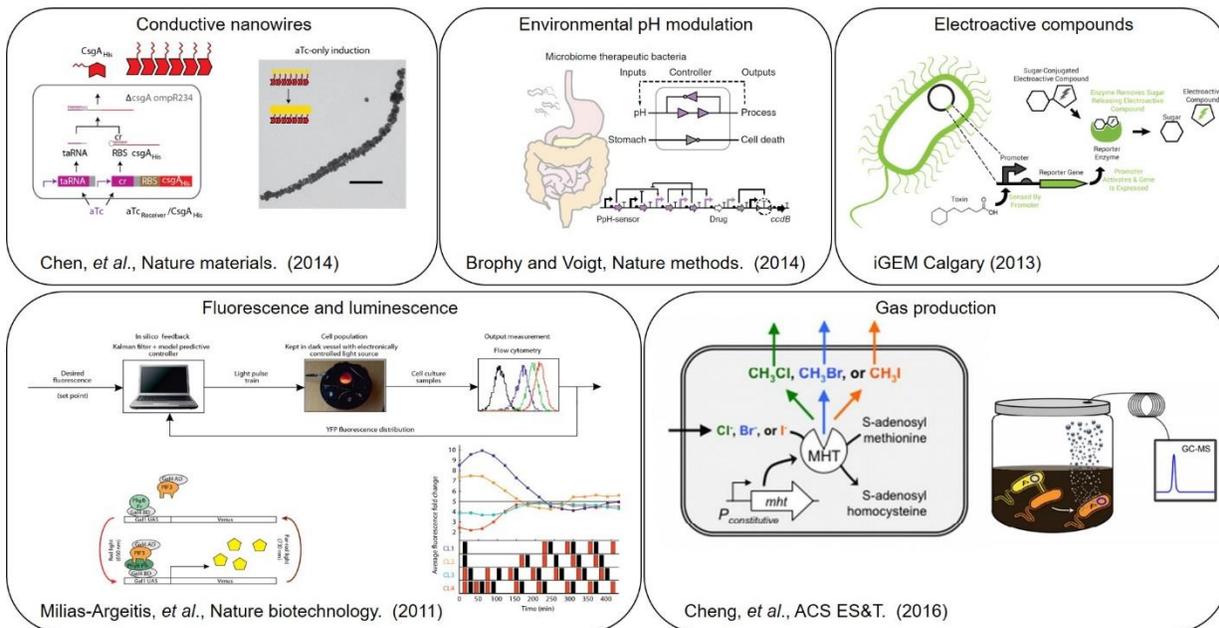


Fig. V. 10. Cells can convert genetic outputs into different physical signals.

However, many existing cell-based biosensors only respond to single or a few stimuli, limiting their use in complex real-world biosensing applications. Thereby, there is an increasing interest in investigating synthetic biology techniques and design methods that can efficiently achieve an array of orthogonal biosensor cells or even engineered cells that can respond to multiple stimuli.

It is shown that both positive and negative selections would be needed to accelerate the evolution of engineered cells and achieve desired sensors (Fig. V. 11). Moreover, alternating positive and negative selection may yield more functional sensors with improved sensitivity, specificity, and dynamic range. Most importantly, leveraging positive and negative selections, one may rapidly optimize the sensor designs and achieve stable cell lines that can respond to multiple different chemical stimuli (up-to 6

inputs shown experimentally), showing a promising and scalable design methodology to support future synthetic biology and cell engineering (Fig. V. 12).

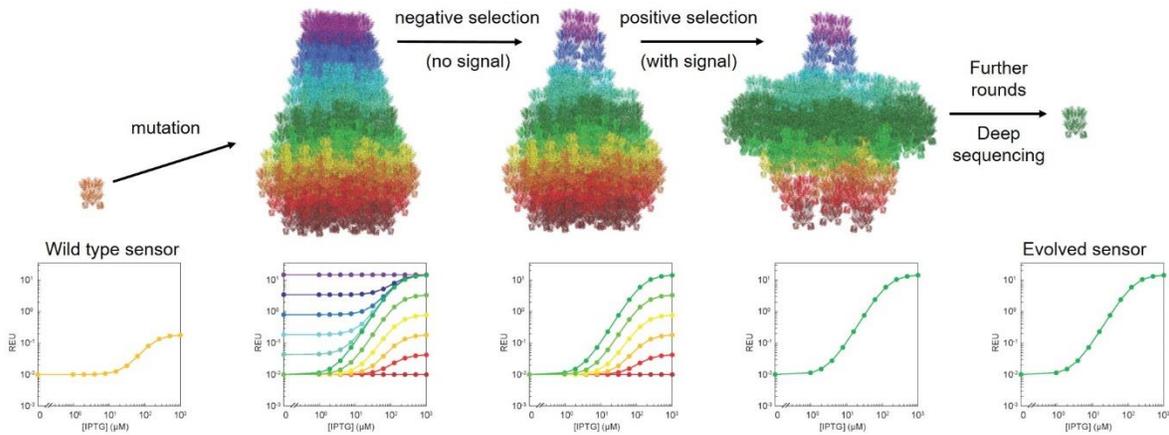


Fig. V. 11. The directed evolution of ideal sensors requires both positive and negative selection.

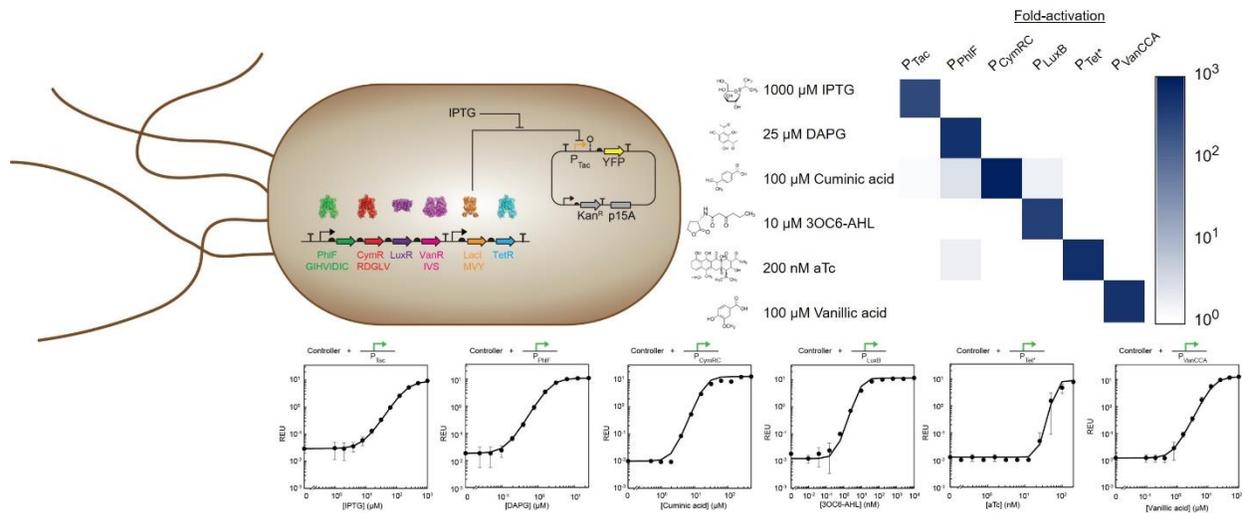


Fig. V. 12. A stable cell line enabling response to up to six different inputs.

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