“Getting a Deeper Sense for Non-Classical Sensing” Workshop Report

Kate O’Neill[1*], Kristopher Murray[2*], Holly Goodson[2], Wolfgang Losert[1], and Larry Nagahara (PI),[3]

Institute for Physical Science and Technology, University of Maryland, College Park[1],
Department of Chemistry & Biochemistry, University of Notre Dame[2],
and Whiting School of Engineering, Johns Hopkins University[3]

[*]equal contributions

Summary:

In September 2019, a two-day workshop, entitled “Getting a Deeper Sense for Non-Classical Sensing,” was held in Washington D.C., that brought together a group of world-class scientific leaders from around the globe to review key findings and progress, as well as new technologies, techniques, and approaches, in the study of non-classical behavior/sensing and its effects on biological function. Participants consisted of researchers who have extensive background with non-classical sensors as well as those who are able to elucidate sensing biological processes or characteristics. Non-classical sensing can occur across multiple length scales and potentially involves collective behavior, which allows for deriving better sensing capabilities from emergent properties in the group, rather than an individual entity. This nascent field of study may allow for potential breakthroughs in understanding, and unprecedented control of biological function and other processes for new applications. The workshop identified gaps and particularly highlighted several potential application areas, including understanding cancer and brain function.

The “Getting a Deeper Sense for Non-Classical Sensing” workshop was hosted by the Johns Hopkins University through grant support by the Air Force Office of Scientific Research (AFOSR) and the National Science Foundation (NSF) and in collaboration with the National Institutes of Health (NIH). Furthermore, the workshop was a follow-on to the “Non-Classical Behaviors in Biological Functions: Potential for Smart Sensing” workshop, held in Arlington, Va. in April 2018 through support from AFOSR. The report below is a summary of the workshop with the agenda and participants for this workshop listed in the appendix.

1.0 Introduction

There is a growing body of evidence of non-classical (*e.g.,* quantum mechanical, biophysical processes, collective behavior, etc.) behavior playing non-trivial roles in a wide range of biological function (*e.g.,* photosynthesis, or magnetoreception) that span multiple spatial and temporal scales. For example, collective motion is observed in many animal species, such as fishes and ants, on the macroscale and subcellular collective behavior is also observed in microtubule dynamics. Just as nature may leverage non-classical behavior to enhance efficiency or functionality, thereby confer a competitive biological advantage, could similar non-classical biophysical effects be leveraged in non-biological systems, and thus create a competitive advantage? Such non-classical biophysical processes can lay the foundation for a new revolutionary class of sensing modalities and open the possibility of "seeing" (and extending) beyond our current detection capabilities in monitoring our surroundings.

Under this basis, a strategic workshop, "Non-Classical Behaviors in Biological Functions: Potential for Smart Sensing," was held on April 12-13, 2018 in Arlington, Virginia. A follow-on workshop, "Getting a Deeper Sense for Non-Classical Sensing," was organized and hosted by the Johns Hopkins University (JHU) through grant support by the Air Force Office of Scientific Research (AFOSR) and the National Science (NSF) and in collaboration with the National Institutes of Health (NIH) on September 23-24, 2019 in Washington D.C. This workshop assembled a disparate group of world-class scientific leaders in the non-classical fields to explore further in depth the following:

- Assess key findings and the progress to date in the field;
- Explore advanced tools and techniques needed to identify new/non-classical behavior and/or provide unmistakable signatures of non-classical effects in biological systems;
- Identify challenges and potential approaches for the incorporation and scaling up of non-classical behavior appearing in smart sensors.

For the purpose of the workshop, non-classical sensing is defined as either sensing of non-classical processes and living systems, or non-classical sensing as it is being applied to measure living systems; and sometimes, it can be both. Over the two days, the workshop was partitioned into eight panel sessions covering the overarching objectives.

2.0 Panel Sessions: Overarching Principles of Non-classical Behaviors

The workshop started by examining some overarching principles in non-classical behavior in a series of in-depth panel sessions around a particular topic (vignette): bio dosimetry, collective behavior, and quantum behavior in biological function. Each session provided an overview of the field from experts and their prospect for sensing.

2.1 Vignette 1: Dosimetry and Biological Function

This session on **"Dosimetry" & Biological Function**, was moderated by **Michael Espey** of the National Cancer Institute. The key take home messages from this session are the following:

- Non-classical sensing would allow us to detect small but consistent changes in biological systems that are likely crucial to biological function (*e.g.,* small fluctuations in the membrane potential of astrocytes);
- Non-invasive sensors will allow us to study interactions between different areas of the body in real time (*e.g.,* the gut brain axis);
- Combining cellular-level sensors with real-time data analytics would enable us to more quickly evaluate treatments (e.g., for cancer) and identify adverse effects of combat (*e.g.,* radiation sensing for pilots).

A more detailed summary of each speaker's presentation and the discussion is included below.

The first speaker, **Valentina Benfenati** (National Research Council of Italy) presented her laboratory's perspective on astrocytes, a type of non-neuronal glial cell that is crucial to producing cognitive function and maintaining brain homeostasis. Benfenati explained how, in order to understand astrocytes' role in the brain, we should consider all the cells in our body as non-classically excitable – as in, not all cell types are electrically excitable like neurons, but many (or even all) cells in the body are negatively charged and have small but real fluctuations in their membranes (Fig. 1). Importantly, these fluctuations can take many forms, whether they are small changes in potential, protein channel expression, or cell shape. Thus, when considering this perspective, the role of astrocytes in modulating brain dynamics becomes clear: astrocytes display significant changes in cell volume during homeostatic functions, and they also exhibit

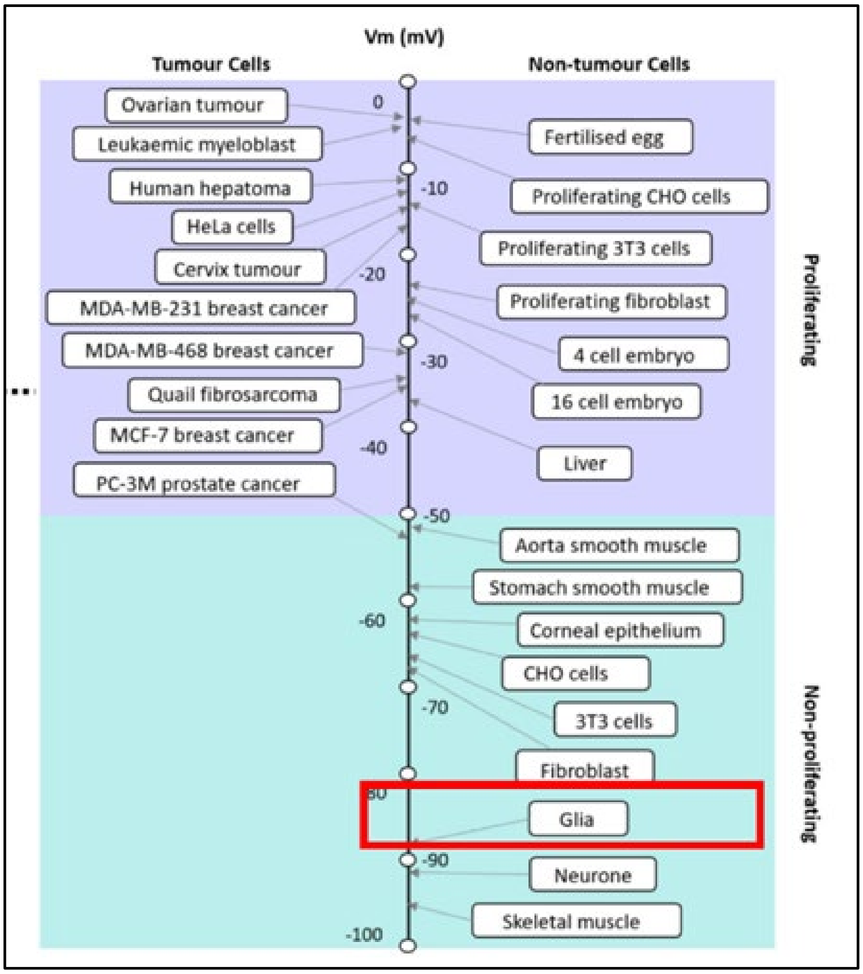


Fig. 1. A wide range of cell-types may be non-classically excitable due to a significant non-zero resting potential. Image from Yang & Brackenbury, Front Physiol, 2013.

slow oscillations in membrane potential as a result of sensing and modulating neuronal activity via tripartite synapses. Importantly, astrocytes accomplish this modulation of neuronal (and therefore, whole brain) activity through exchange of ions and water molecules in the peri-synaptic space (Fig. 2). The importance of these physiological activities was previously ignored. Moreover, astrocytes' regulation of neuronal activity causes the astrocytes in turn to have their own activity: slow-wave oscillations that appear to modulate higher-order functions in the brain, such as decision making. Considering this unique perspective on biological function, Benfenati closed her session with some important questions:

1. Since astrocytes have their own slow rhythms, do they contribute to the whole brain oscillations observed in EEG and other brain imaging modalities?;
2. Does the flux of water and ions throughout the brain modulate the slow-wave activity of astrocytes?;

3. Given astrocytes' ability to sense and respond to their external environment, might they even be able to function as a type of quantum biological sensor? These are all important questions to consider as we become more attuned to the non-classical view of biology.

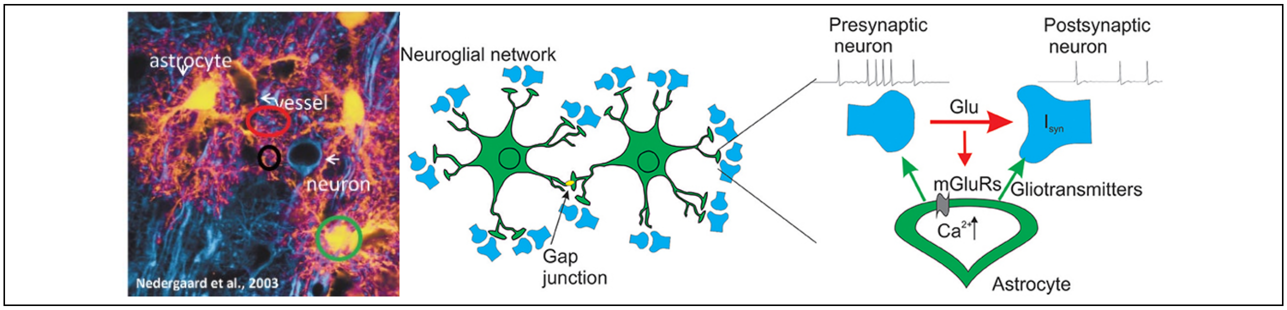


Fig. 2. *Networks in the brain include astrocytes as collective signal integrators.* Image on left: Image adapted from Nedergaard et al., 2003. Image in center and on right: Image from Gordleeva et al, Front Physiol, 2019.

Jeffrey Buchsbaum (National Cancer Institute) delivered the second presentation of this session and discussed the effects of radiation on cancer cells. Buchsbaum brought a non-classical perspective to the dosimetry part of this discussion by explaining that treatments are usually prescribed in Joules/kg of the person and that outcomes have traditionally been assessed based on the size (usually in mm) of a tumor. However, both this type of pre-treatment prescription and post-treatment assessment do not consider the heterogeneous responses of tumors to the radiation. He added more nuance to this perspective by further clarifying that what is important to investigate is not just whether a certain cell type is susceptible to radiation but also what characterizes its genetic response, especially to low doses of radiation (Fig. 3). While the traditional treatment plan would provide several weeks of treatment and then require several months before assessing outcome, Buchsbaum asserts that this method of waiting is antiquated and that there should be a way to evaluate the effects of radiation much more quickly, ideally in real time. Essentially, Buchsbaum advocated for the dynamic use of information for assessing treatment rather than using preset timepoints that only offer a snapshot of the effects of dosimetry. Such dynamic information would allow physicians to modify treatment plans for patients within days rather than months. In particular, the ability to understand with greater temporal resolution how radiotherapy affects a tumor has the potential to change a patient's status from resistant to radiotherapy to successfully treated by radiotherapy. What this would require, Buchsbaum suggests, is the development of non-classical sensing tools that would allow physicians to measure biological responses rapidly. The ability to gather this type of data would also greatly improve computer models since they often cannot predict all the complexities of the biology. Finally, Buchsbaum poses the following question: how do we develop technologies to allow for non-invasive measurement of biological responses to treatment in real time?

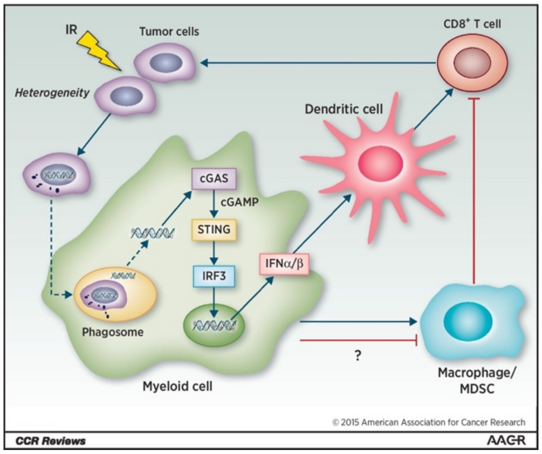


Fig 3. *EM radiation can trigger cell signaling within and among cell types during cancer treatment.* Image from Deng et al, CCR, 2016.

The third presentation was given by **Bianxiao Cui** (Stanford University), who discussed technological advances for sensing bioelectric potentials. Building off Benfenati's explanation of the neuro-centric view of brain activity, Cui explains that while the brain is usually the subject for analysis of electrical activity in biology, bioelectric potentials exist in places as seemingly unlikely as bacteria, which is most definitely a non-classical view of biology. Cui then reviews the development of bioelectric sensors, which started with traditional, electrode-based methods and has expanded to include optical-based methods. Moreover, Cui explains that optical sensing methods were developed as a result of the tradeoffs of electrode-based sensing: superb temporal resolution is achieved at the cost of a fixed location and low spatial resolution. Optical-based sensing methods have also matured from fluorescence-based modalities to label-free modalities and have recently started to include material-based sensors. A great example of a materials-based sensor is the nitrogen-vacancy centers in diamonds, which allow for label-free sensing of bioelectric potentials, but due to the low sensitivity, detection can only be achieved with evoked potentials where the signals can be registered in time. While there is room for improvement, Cui suggests that these electrochromatic materials may be the future of label-free, optical sensing of bioelectric activity due to their myriad advantages. She demonstrates how electrochromatic materials accomplish sensing in a variety of situations, including immortalized cell lines, primary cultures of dorsal root ganglion neurons, and even whole slices of hippocampal neurons. Finally, Cui ends her presentation by asserting that two-dimensional quantum materials have the potential to be the future for optical sensing of electrical activity.

They allow for fast, sensitive detection of action potentials in a label-free manner in a variety of cell types, and even more importantly, they would allow for the detection of sub-threshold membrane potentials (*i.e.*, not action potentials), which Dr. Benfenati previously discussed could have significant biological importance.

Building on the discussion of brain activity, **Read Montague** (Virginia Tech University) began the fourth presentation of the session by discussing how functional magnetic resonance imaging (fMRI) has been used to infer neuronal activity by measuring metabolic demand. Montague reviewed the advantages and disadvantages of fMRI, explaining how the non-invasive nature of it is balanced by its low spatial and temporal resolution. Moreover, this is a very indirect measure of neuronal electrical activity. He then reviewed the development of other non-invasive imaging modalities, such as magnetoencephalography (MEG) and magnetic measurements using rubidium. The state of these technologies is similar to fMRI in that they present significant limitations in terms of sensitivity to movement and low spatial resolution. Montague then explained some of the advantages of more invasive voltammetry measurements, such as when electrodes for Deep Brain Stimulation (DBS) are implanted into Parkinson's Disease patients. Harkening back to Cui's presentation, electrodes have significant disadvantages that cannot be mitigated, such as their fixed spatial location. Montague then put the complexity of the brain into perspective for the audience by explaining how the brain has three miles of wiring for every cubic mm of space, with each synapse releasing neurotransmitters. For all the advances in computing power and artificial intelligence, we still do not understand nor can we yet mimic this style of computation. While we know about and are very aware of the brain's electrodynamics, we are less attuned to the magnetic dynamics and chemical dynamics, as other speakers have suggested. It is necessary to develop better, non-invasive imaging modalities that would allow us to detect these types of non-classical dynamics. Finally, Montague ends his presentation with the following questions:

1. How do we approach the multiscale problem of measuring delivery of chemicals (neurotransmitters at the synapse) while a human being is performing a task?;
2. Assuming we can detect these non-classical dynamics, can they be used for detection of quantum biological events?

The last speaker of this session is **Theodoros Zanos** (Feinstein Institute/Hoftstra School of Medicine), who opens his presentation by explaining how the brain is not an isolated organ but rather interacts with the rest of the body. Zanos's particular focus is on the gut-brain axis and how bioelectricity in the nervous system may influence behavior in the rest of the body, particularly in pathophysiological cases. His example of this is the vagus nerve, which connects the brain to several organs (heart, kidneys, lungs, liver, etc). Most of the tracks (approximately 80%) within this nerve are afferent, meaning they bring sensory information from the organs to the brain. While the current state of knowledge recognizes that this exchange of information occurs, we have a very limited understanding of the nature of this signaling. Is the signaling binary, as is the case with action potentials, or is the signaling more nuanced? The most challenging part of investigating vagus nerve signaling is the inability to interface with and to gather information long-term from this nerve in a chronic model. Due to this limitation, we also do not understand the diversity of the molecular and chemical signaling sources. What is needed to interface with the vagus nerve and similarly complex biological systems is to build devices that are compatible with the central and peripheral nervous systems and that allow for the detection and modulation of the nerve signals. What is known is that the signaling dynamics occur across multiple spatial scales, especially related to inflammatory events occurring at one or multiple organs. Zanos suggested one way for this communication to occur efficiently is if field potentials are synchronized across scales. Moreover, he considers the importance of metabolic states in this information flow. For example, how is blood glucose level detected and sent in an electrical signal back to the brain? There are currently no tools for detecting this signaling event reliably. Finally, considering the potential quantum nature of such events, he suggested that ion channel coherence may even play a role. This presentation has highlighted the untapped potential of utilizing the nervous system to diagnose and treat diseases through electricity.

The discussion and question and answer period were opened with a request to think about how we can leverage information gathered at the cellular level to understand complex events, such as organ function or cancer progression.

The first question considered the non-classical nature of the brain. From the discussion, the audience learned how the process of learning in the brain is conserved at every level that has been investigated, from network states to the organelle level. The idea of maintaining coherent states from nm to cm is as non-classical as it gets. Moreover, the flux of molecules, such as ions and water, are atomic/molecular level events that clearly have organ-level effects. The second question focused on whether it is possible to detect quantum events in the brain. It was discussed that the quantum effects at the molecular level seem to be lost at the organ level. The consensus was that the brain is not entirely quantum, but maybe we can refer to the techniques we use to understand the brain as quantum. The discussion then moved towards wondering whether the brain uses information about mm-scale movements that characterize autonomic physiological functions (breathing, heartbeat, digestion, etc.). Does the brain or body use this information, or is it just biological noise? The consensus was that we do not fully understand how this information processing occurs and that it would be interesting to know how to filter out this much noise. The discussion then moved to other aspects of brain activity, such as the ability to model all bioelectric signals in the brain and not just those that can be traced to neurons. The ability to record long-term is most essential to answering this question and will illuminate the role of other cells in the brain (not only neurons) because the difference between a human and rat brain cannot only be explained by neurons. Rather, there are other cells, like astrocytes, that play critical roles, and these functions are likely more complex in a human compared to a rat. Moreover, while neurons have the most easily detectable action potential – i.e. they are the "loudest" – but that does not mean that they are even the most important cell. The closing discussion highlighted how structure and function are intimately related but also that some of the ways we have been trying to achieve sensing may inadvertently alter the function of cells because they have mechanical memory. Non-invasive sensing will eliminate this need, at least at the cellular level, but may still have quantum effects that alter function.

2.2 Vignette 2: Collective Behavior

This session on **Collective Behavior**, moderated by **Holly Goodson** from the University of Notre Dame, consists of the of the following key take home messages from this session are the following:

- Non-classical sensing not only can occur across scales, but also can connect phenomena across those scales;
- Lessons from biological systems can be applied to man-made systems to make them more effective and more comparable to the systems by which they were inspired;
- Many examples of collective sensing behaviors have already been observed and characterized in nature, with examples at scales from the cellular (*e.g.,* excitable membranes) to the microscopic (*e.g.,* quorum sensing bacteria) to the macroscopic (*e.g.,* ants, fish).
- Excitable systems can rely on both cues from reaching a quorum number and cues from spatial arrangements;
- Excitable systems that lie near the boundary of a phase transition are ideal for inducing non-classical behaviors.

Holly Goodson (University of Notre Dame) also kicked off the session and broke her presentation into three 'vignettes' to illustrate how collective sensing behaviors in the environment can occur across scales via different mechanisms but lead to similar results. She stressed that collective behaviors are particularly effective at locating resources or threats and allowing groups to detect and compare levels of sensors that would be difficult for the individual.

Goodson's first vignette discussed how bacteria can use biased-random walks to identify gradients of an attractant in their environment as autonomous individuals whose movements are coordinated through local communication. Her first example of this was bacterial chemotaxis, a classic example of bacterial behavior where swarms of bacteria utilize a positive-feedback loop to bias their movement towards a chemoattractant. Her other example was quorum sensing in bacteria, where once a quorum (the minimum number of members required to make a decision) is reached, a particular behavior is triggered (*e.g.,* light production) by that group of bacteria (Fig. 4). These methods are simple and robust, but sensitive enough to be effective. Her second vignette was inspired by work from Deborah Gordon at Stanford. She discussed how ants explore randomly for food and leave chemical trails to indicate to future ants the paths that lead to food. This allows them to find targets by using an expendable resource (worker ants) to create a robust network and provide an opportunity for communication amongst the ants across time. In Goodson's final vignette, she discussed fish schooling behavior, work that was inspired by Iain Couzins at the Max Planck Institute of Animal Behavior: fish are able to avoid danger by working together with members of their school. Though they are unable to sense resource gradients as individuals, by observing the density of their neighbors, fish in groups can find or avoid targets using simple rules and do this more effectively while in school than as individuals.

Goodson concluded her presentation by reviewing applications of collective behavior and used the example of drone swarms. Current methods of drone coordination require direct wireless communication, which is unreliable and potentially vulnerable to outside influences. However, she discussed research suggesting that coordination between drones could be more effective if they were allowed to have more autonomy to mimic behaviors seen in biology and allow collective behaviors to evolve through that autonomy. This could allow for the optimization of behavior in any space and be generalizable across scales.

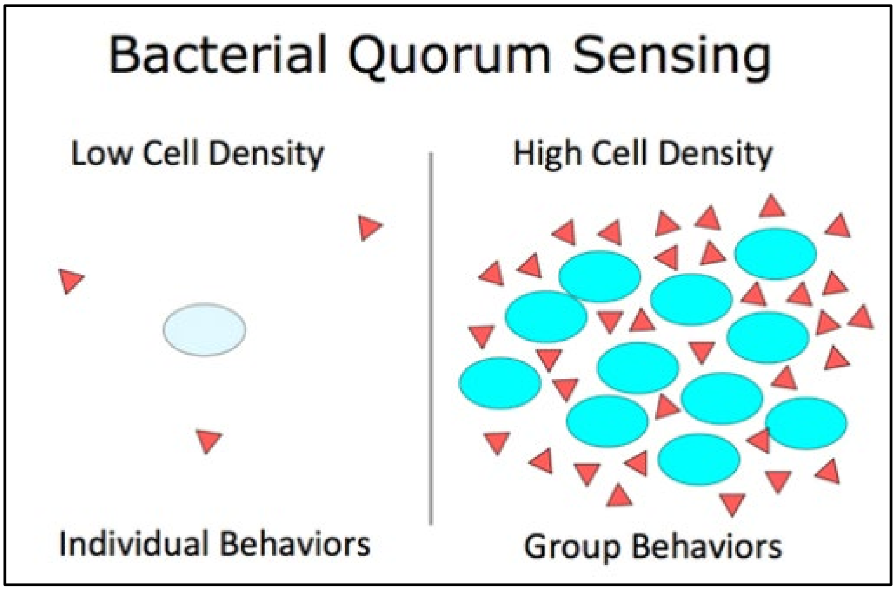


Fig. 4. *Quorum sensing allows simple cell types to exhibit group behaviors. Image from* https://scholar.princeton.edu/basslerlab/research

Sylvia Daunert (University of Miami) discussed the coordination of collective behaviors in the human microbiome. The microbiome is a fundamentally collective system, and different regions of the body have unique bacterial compositions. She stressed that though we can identify the different bacteria in the microbiome and do metagenomics to identify the levels of transcripts, metabolites, proteins, and also map interactions of these components, this alone cannot give us any information about how the microbiome system will behave as a unit.

Daunert focused her presentation on quorum sensing in the microbiome, and how this simple method of communication can lead to a variety of different complex collective behaviors. Her lab has developed biosensors to allow for the analysis of quorum sensing behavior in *E. coli*. She focused on the interaction between the microbiome and the host and the intricacies of that relationship. Both individual bacterium and the groups of bacteria interact with the host. She used the two faces of Janus, the Roman God of Beginnings, to illustrate the duality of the body's relationship with bacteria. Daunert finished by discussing the role of the neurotransmitter serotonin and whether it could have an impact on quorum sensing. She showed that serotonin can be recognized by bacterial quorum sensing systems, but also can induce quorum sensing behavior in bacteria without these systems to induce the production of virulence factors and the formation of biofilms. She concluded by proposing a variety of discussion questions about how quorum sensing in the microbiome can be better understood and utilized by researchers and clinicians.

Fig. 5. *Collective behaviors are likely important for the physiological relation between humans and microbes and for understanding this relationship (https://www.nature.com/collections/scqssjswcq).*

Wolfgang Losert (University of Maryland, College Park) discussed spatial patterns of organization in collective behavior and how this is essential for *in vivo* systems. He focused his presentation on how collective behavior emerges in *Dictyostelium discoideum*, which allows individuals to identify other *Dictyostelium* to form self-organized fruiting bodies for protection. He described these behaviors as excitable systems, where signals in distinct dynamic states dictate those behaviors. He then described a more familiar excitable system, the brain, where cell behavior can respond strongly to small perturbations in the form of electric oscillations or waves that can be self-sustaining. These excitable behaviors can be seen in purely chemical systems by observing the biomechanical waves of actin propagation in *Dictyostelium*: the cells use these biomechanical oscillations to sense their environment. Losert discussed how the behavior of the actin excitable system can be modulated with weak electric fields to change cellular behavior, without the need for any genetic changes. He concluded by discussing challenges in studying excitable systems, particularly in finding ways to control them by coupling them at one scale or linking systems across scales.

Shashank Priya (Pennsylvania State University) finished the session by discussing technological tools that can be used for imaging. He first focused on magnetic field sensing, which couples ferromagnetic materials and designed ferroelectric materials and allows for interactions with the environment. This produces a voltage output that can be measured, but measurement is difficult due to reasons such as low signal to noise ratios and the lack of cooling mechanisms. Magnetic field sensing, however, provides an opportunity for sensing room-temperature environments at low cost and could be particularly effective for non-invasive dynamic brain scans through magnetoelectric sensors. He indicated this technology has great potential and could be developed for multi-scale imaging. Priya then transitioned his talk to a discussion of motion. He described pill-cams that are swallowed to allow for the visualization of the GI-tract and how he is developing ways to give these pills locomotive capabilities. This would allow the pills to be highly mobile in the body and give researchers the capability to more effectively visualize the GI tract and potentially allow for the collection of samples at sites of interest. He finished by describing the locomotion of millipedes, whose evolutionary design ensures that there are legs on the ground at all times, which allows for movement in a variety of different environments. He has designed robots inspired by millipedes; whose legs oscillate on a duty cycle that allows them to move on a variety of different surfaces.

The Q&A session was opened by an attendee wondering why collective behavior is important to study when it seems like the collection of a large number of sensors in the system is what is key to the effect. He asked whether nonlinear effects had been observed to influence the phenomena mentioned by the panelists. Holly Goodson made the point that the whole is greater than the sum of its parts in these systems and that the key to the number of sensors makes the system robust to noise. Wolfgang Losert added that collective behavior allows for a certain level of precision and accuracy from noisy individuals that would be difficult to replicate. Losert also added that little is known about how emergent phenomena are linked across scales but that studying collective behavior could fill in those gaps. Sylvia Daunert agreed with the attendee's point and pointed out that both the number and positioning of the "sensors" are key to collective behaviors.

Jeffrey Buchsbaum asked how this information could be utilized in a volumetric system, for example by looking at an entire body. Holly Goodson pointed out that it would be difficult to study an entire body, but noted that the immune system is a good example of collective behavior, as you have space exploration occurring physically by immune cells and also the exploration of sequence space by a constantly adapting immune response. Buchsbaum countered by pointing out that there is a school of thought that cancer is a cause of the failure of the immune system, and asked what the panelists believed would happen when systems of collective behavior fail? Goodson replied by pointing out that cancer is also undergoing space exploration independently from the immune system, making it difficult to determine which changing system (the immune system or the cancer cells) will overcome that failure first. Losert also added that although systems are typically measured in 2D for practical purposes, there is no reason to believe that there are not three-dimensional aspects of already observed collective behaviors.

Valentina Benfenati noted that shape and structure is key for generating proper collective behavior, and wondered if there is any information about collective behavior in the brain. Losert answered by giving examples of collective behavior in the brain that he has studied, and wondering aloud if electrical excitability in the brain could be harnessed as a collective behavior.

Michael Espey asked if there was a way to amplify signals of collective behaviors, and if a sensor could be built that amplifies signals. Losert hypothesized that a sensor could be built that harnesses electric fields to amplify collective behavior signals. Losert and Daunert proposed a sensor that could identify the beginnings of collective behavior could be particularly effective at studying cancer cells. Shashank Priya also pointed out that stress-based sensors are known to be particularly effective when studying systems near a phase transition.

2.3 Vignette 3: Quantum Behavior in Biological Functions

This session on **Quantum Behavior in Biological Function**, moderated by **Wolfgang Losert** of the University of Maryland, College Park, consists of the of the following key take home messages:

- Surface chemical potential gradients can enhance the functioning of nanosensors by aggregating analytes;
- Vibrational coherence may enhance the ability of bimolecular systems to sense;
- Magnetic quantum transition may enable control of ROS reaction pathways, and allow for exploring the emergent properties of quantum chemistry on metabolic pathways.

Paul Brumer (University of Toronto) started the session with a discussion of quantum coherence and dynamics in biological processes. The focus was on what Brumer identified as “non-trivial” quantum effects associated with vision. The specific focus of the presentation is on coherence, since classical mechanics involves the destruction of coherence. Brumer highlighted studies of vision – a signaling process that includes multiple non-classical features. One example highlighted by Brumer is the vibrational coherence of the dynamics involved in the vision cascade.

Henry Hess (Columbia University) described nanoscale sensing enabled by quantum chemistry. Hess started by noting that quantum chemistry effects generally get averaged out rapidly even on short timescales. Then he introduced several non-classical dynamical processes in living systems, including swarming and active transport. The bulk of the presentation focused on directed transport of molecules by chemical potential gradients that enhance analyte collection in sensors. Hess demonstrated one of the consequences of "non-classical" biochemical reactions is that they exhibit non-exponential behavior.

Robert Ussellman (Florida Institute of Technology) introduced quantum redox switches in his presentation. The underlying physics of the presented non-classical effect is that the radical pair spin dynamics affect the reaction pathways, and thus reaction pathways can be influenced by magnetic fields via Zeeman energies and hyperfine coupling. Through such spin-driven partitioning of ROS, the presenter proposes the ability to alter steady state levels of oxygen and hydrogen peroxide.

The final speaker of the vignette, **Carlos Martino** (Florida Institute of Technology) expanded on the prior presentation and presented on coherent transition pathways, focusing on how to drive biological systems into controlled quantum states with controlled magnetic fields (Fig. 6). He introduced the challenge that the coherence of such states is short, on the order of nanoseconds, often too short to influence the dynamics of the system. The objective of the presenter's research is to control the product yield by mathematical analysis of the spin Hamiltonian. The presenter discussed the opportunity to drive the system with complex spatio-temporal magnetic fields that may optimize product yields.

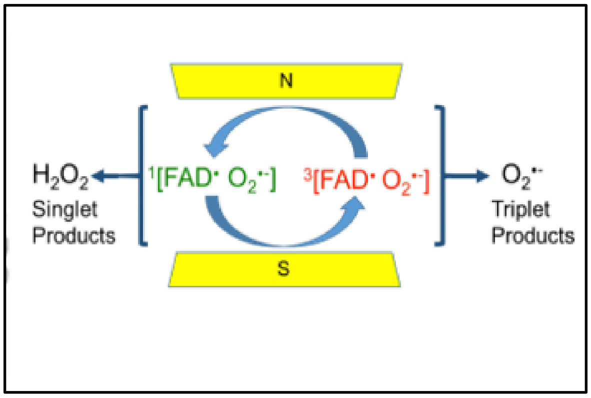


Fig. 6. *Oxygen metabolic pathway involves quantum states that may be modulated with magnetic fields. Modulating the quantum state has the potential to alter metabolite balance.*

3.0 Panel Session: International Research Experiences

Shashank Priya (Penn State University) moderated the session and discussed how the need for biosensors has increased around the world from applications related to agriculture, medicine, and food distribution, to name a few. Chenzhong Li at NSF leads this effort and is one of the Editors for Biosensors and Bioelectronics. The growing market share for biosensors is increasing on the order of several tens of millions of dollars per year. Regarding international collaborations, Priya describes how a U.S.-Australia Joint Commission Meeting took place several years prior that lead to leading universities across the country, including Penn State, Johns Hopkins, and Texas A&M to establish an international partnership in biosensing that has grown to include countries such as Italy and South Africa. This led to Penn State launching an International Institute for Biosensing (IIB) that initially started with 8-9 institutions but has now grown to linking a network of networks that include over 50 institutions spanning 14 countries. This is one example how international research can flourish from a grassroots effort. The session is more about: 1) how we could develop some playbook, guidelines, and policy; 2) what are some of the barriers and challenges of international collaborations; and 3) what are the promising ways to move forward and accelerate these collaborations.

Giulio Busulini (George Washington University) described some of the activities that occurred over the past years. Previously, Busulini was a diplomat at the Embassy of Italy in Washington D.C., where his role was the Science and Technology Counselor. His mission was to work with U.S. federal agencies, such as AFOSR, NSF, NASA, etc. During that time and now, he commented on how these partnerships have blossomed and are starting to bear fruit not only with U.S. researchers, but with other countries, such as Australia. Partnerships are very relevant and allow for researchers from each country to better leverage their country's own funding agencies, but also grow internationally. For example, opportunities within Europe through these connections allow for joint projects with the European Union (EU) Commission funding. One of the key ingredients for the success is AFOSR's willingness to be open with a vision of international collaboration by reaching out and engaging with Italy. From that start, this grew to collaboration with multiple agencies. From his current position at George Washington University in the Office of the Vice President for Research, he talks of the importance to engage with industry early so that in several years' time the relationship is built and capable of transferring that knowledge/discovery into a useful application. An example in the U.S. is the NSF I-Corps program that helps researchers gain valuable insight into entrepreneurship, starting a business, or industry requirements and challenges.

Nicole Forrester (Commonwealth Scientific and Industrial Research Organization (CSIRO)) described the CSIRO and the collaboration the organization has with the U.S. The fact that Forrester is on assignment in Washington D.C. is a testament to the importance of international collaboration between the U.S. and Australia. With approximately 5,000 scientists in the organization, many carry multiple roles. For example, Prof. Sally McAuthur has a joint appointment in the Department of Biomedical Engineering at Swinburne University and is also CSIRO OCE (Office of the Chief Executive) Science Leader whose role focuses on the development of 3D tissue model systems as new in-vitro test platforms (with biosensors) for the biomaterials, pharmaceutical and medical/bio technologies sectors. Forrester's role is to be a connector with U.S. researchers to identify potential partners. While not a funding agency, CSIRO is an Australian science agency receiving two-thirds of their funding from the Australian Government and specializes in taking basic science and translating it into more applied science. Next, she discussed the Australian Centre for Disease Preparedness (ACDP) (formerly Australian Animal Health Laboratory), which is a high-containment facility designed to allow scientific research into the most dangerous infectious agents in the world. What make the ACDP unique is its advanced technology and infrastructure, allowing research and diagnostics that require the highest level of biosecurity and biosafety within a laboratory environment. One of the other accomplishments from CSIRO is Wifi, but regarding this workshop, CSIRO has put sensors on thousands of honeybees to monitor the insects and their environment using a technique known as 'swarm sensing'.

Franklin Carrero-Martinez (National Academy of Sciences) discussed his previous experience in the Office of International Science and Engineering (OISE), his time at the U.S. Department of State and his current position at the National Academy of Sciences. His perspective is from a science policy angle. He offered the following suggestions for starting a nascent field in science: 1) The community needs to have something out in the scientific community so that the agencies (AFOSR, NSF, NIH, etc.) can point to and say this is something worth funding and a community out there that will push this field forward. 2) The science behind the nascent field needs to be "repackaged" for the general public. Carrero-Martinez gave his own personal account where his mother had cancer which is in remission. However, she's not 'cured' as there's no technique for determining that a person in remission is truly is 'cancer-free'. So, the outcome from this workshop could have the potential to spur a technology that can definitively define for a doctor whether a patient is 'cancer-free'. The same could be true for other diseases like AIDS and hepatitis. There's a huge need for biological sensors for environmental monitoring and sustainability. Policy makers also like to hear about the economic angle – how many jobs this will create; how it benefits the economy.

Lastly, researchers have a powerful voice to say something to policy makers about international collaborations.

Misoon Mah (Air Force Office of Scientific Research) opened her discussion with how AFOSR is a unique organization that manages basic research (6.1) for the Air Force both domestic and international. AFOSR has done a lot of international collaborations by supporting currently approximately 400 grants to 43 countries. Window on Science (WOS), which brings foreign researchers from all over the world to meet with AFRL scientists and engineers to share their research, is one example to promote international collaboration. Dr. Mah next talked about the need for data analytics to examine more deeply the international component in non-classical sensing and use that information in planning for the next workshop. She pointed out that approximately 75 percent of the overall global R&D expenditure is conducted outside the U.S. and that share is growing rapidly.

Yuko Tsuda (Japan Science and Technology Agency (JST)) explained the role of JST in funding fundamental research in Japan. In more recent years, Japan is emphasizing more international collaborations and the need for more science & technology diplomacy as a result of rapid globalization. There are many areas of research, such as environment, energy, natural disasters and human diseases, which no one country can solve and requires a global solution. For a country like Japan to maintain its science and technology, it needs to rely on more international collaborations. JST manages one program called Strategic International Collaborative Research Program (SICORP). Based on intergovernmental agreements, JST executes international joint research in research fields that contribute solutions to challenges facing the world today. JST provides large-scale funding to Japanese researchers though SICORP and counterpart researchers are to obtain project funding approximately equivalent to SICORP from funding agencies in their respective countries.

Panel Discussion: A common complaint by scientists around the globe is the need for government to put more funding into research. So, while this task will always be there, the non-classical sensing community needs to make a strong argument for why this particular area of science can not only further our understanding, but have an impact on industry and society and thus be prioritized higher than some other field.

Over at the NIH/NCI, funding is available, but is becoming more competitive. Nevertheless, international collaborations on these awards are on the rise as well and therefore funding mechanisms are in place for including foreign collaborators on these grants. The bigger need for international collaboration seems to have the foreign funding agency to provide smaller seed programs so that they are more competitive to go after the larger awards.

CSIRO and NASA are in collaboration together through one of its programs that might be relevant to the workshop participants.

NASA’s Translational Research Institute for Space Health (TRISH) is releasing a new funding initiative supporting research advances in the study of effects of space radiation on human physiology and seeking countermeasures to be used in deep space exploration.

AFOSR has a similar mechanism described by others on this panel for bilateral funding between two government agencies.

4.0 Panel Session: Manufacturing on Smart Sensors

Larry Nagahara (Johns Hopkins University) moderated the session, and the initial discussion was led by **Zhijian (ZJ) Pei** (Texas A&M). Pei talked about his time at the National Institute of Standards and Technology (NIST), Advanced Manufacturing National Program Office and previously at the National Science Foundation (NSF) where he was a program director for the Manufacturing Machines and Equipment Program. While in these positions, he talked of the promise of 3D printing as a means to bridge smart sensor manufacturing on a large scale. Other potential advantages that 3D printing could bring to smart sensing is fabricating novel, complex designs that are cost prohibitive using traditional 2D (planar) printing.

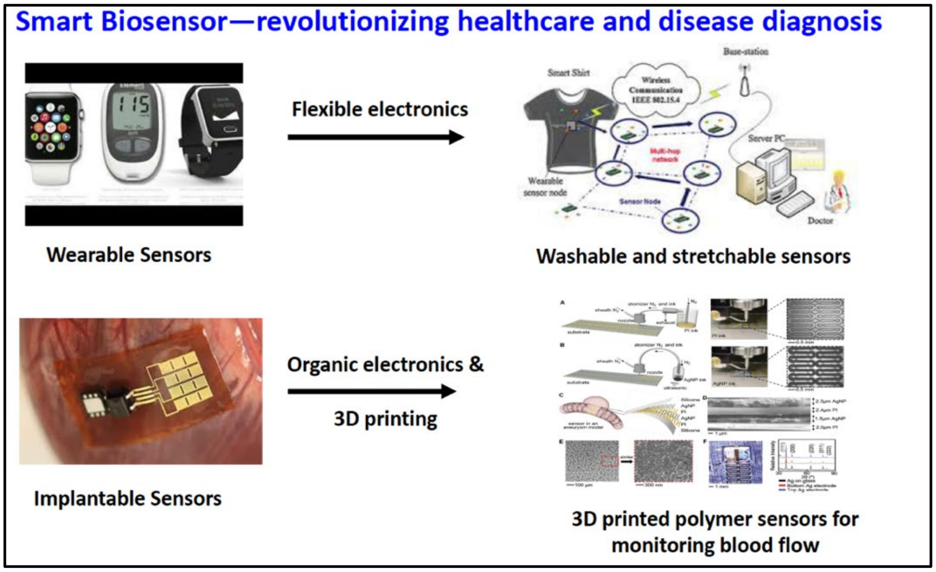
Kaiming Ye (Binghamton University) started with the prospects of moving away from the traditional enzyme-based glucose sensors toward a long-term, minimally invasive, implantable biosensor. Using a Förster (Flourescence) Resonance Energy Transfer (FRET) based sensing platform has the advantage of minimal background noise and is easily detectable in the physiological glucose concentration level (mM). Moreover, the sensor platform is stable for many weeks (> 5 weeks).

Next, Ye discussed the development of smart biosensors for applications beyond medicine and healthcare. The massive deployment of sensors can greatly affect the way we produce food through incorporating smart sensing in agriculture as well in monitoring the safety of our food supply chain. In addition, environmental monitoring with smart sensors will play an increasingly important role in the coming years.

Besides the detection platform, key considerations for smart sensing need to include power and wireless (data transfer) capability.

One promising area where smart biosensors can lead to revolution in healthcare is the incorporation of flexible electronics (Fig. 7). For example, designing (multi) washable and stretchable biosensors into the fabric of clothing will allow for long-term monitoring. This is a supported project by the Department of Defense (DoD). A subarea within the flexible electronic field is to move away from using metals and to incorporate organic electronics for facile manufacturing whether it be ‘reel-to-reel’ or novel 3D printing. Flexible electronics also allows for the ability of generate power, without the need for a battery, by incorporating piezoelectric materials.

Some of the challenges for manufacturing implantable smart sensors are biocompatibility in either being biodegradable or easily retrievable. As a living body is never still (motionless), designing smart sensors that will be flexible and stretchable are important design parameters. Lastly, while there are existing industrial standards for physical and chemical sensors, in the case of printable biosensors there are no established standards. However, Ye is working with the federal government to incorporate such standard in regenerative medicine.


Smart Biosensor—revolutionizing healthcare and disease diagnosis

Wearable Sensors

Flexible electronics

Washable and stretchable sensors

Implantable Sensors

Organic electronics & 3D printing

3D printed polymer sensors for monitoring blood flow

Fig. 7. Considerations involved in the development and manufacture of smart sensors. Advances in flexible electronics, organic electronics, and 3D printing are starting to facilitate novel applications of smart biosensors.

Testing of biocompatible materials using 3D printing was discussed as a means of advancing the field forward for advanced manufacturing of smart biosensors. In addition, there is a fine balance between the customization (e.g., patient specific or detection of specialized/rare disease) that 3D printing affords and the mass production (population) scaling of traditional manufacturing.

During the discussion, the question of reproducibility of biosensors was raised and the need to have reliable, manufacturable materials that allow for more controllability. In contrast, polymer-based materials used as scaffolds from 3D printers are much less reliable. Moreover, in-vivo use of polymers has a lot of uncertainty, as most implantable materials tend to be non-polymer based. This difficulty is amplified by the inability to perform (re)calibrations of the biosensor while the sensor is implanted inside the body. Next, a question arose about the viability of using protein-based sensors as a long-term monitoring device. For short time/immediate use case, protein sensors have proven to be quite valuable as they are a very good platform for specificity, but they tend to degrade quite rapidly in the presence of liquids (e.g., water). The discussion turned to ex-vivo system, where the challenges of in-vivo monitoring are reduced by building sensors that could be initially tested for ex-vivo environments before being incorporated as an in-vivo sensor. The cycles of troubleshooting in an ex-vivo system will be much faster than going directly into an in-vivo system. Lastly, the discussion ended on modifying one's own cells to act as a sensor to monitor bodily function: for example, an immune cell modified internally with a sensor to probe the body as it moves around. The issue here is a readout form to the outside world. In nature, when an immune cell detects something, the communication might be in the form of attracting more immune cells as a collective behavior. However, in a modified cell sensor, what macroscopic signal could be used to inform either the host or outside of its finding? Moreover, it should be taken into account that individual cells from the same cell line may not respond identically. The same could be asked if these same cells would behave identically a year later. As an example, two nearly identical cell (or media) types from a biochemical and cell signaling perspective provided a very different response under a shear flow condition. Thus, it is cautionary, especially if the signal detection is non-classical, to ensure that such cell-based sensors are reproducible.

5.0 Panel Session: Optical Probing Non-Classical Phenomena

Michael Espey moderated the session. **Arkaprabha (Arka) Konar** (Kent State University) started with revisiting the definition of non-classical sensing/phenomena from the first workshop (18 month prior). From a physics perspective, phenomena that can't be described with classical physics, such as quantum behavior, found in atoms, up to macromolecules. The perspective that the act of measurement affects the system (*i.e.,* leads to decoherence) and the quantum effect is relatively short lived. From the biology perspective, non-classical phenomena can be viewed as emergent behavior in a living system, such as collective behavior.

Konar described the recent announcement by Google on achieving “quantum supremacy” with the company’s Sycamore Processor. Being able to demonstrate quantum computing speed supremacy over classical computer is a major milestone. He talked about the Sycamore Processor solving a particular problem in mere 200 seconds, which would take the IBM Summit – one of fastest and largest supercomputers – 10,000 years to complete. By harnessing the “quantum-ness” of a system, one might achieve tremendous advancements, but Konar cautions that one critical need is to maintain quantum coherence over an extended period (minutes). One way to achieve this is to go to ultralow temperatures.

With regard to using optical probes to interrogate biology, Konar described a canonical paper of isomerization of rhodopsin molecule, as well as in an engineered synthetic molecule. Moreover, non-linear spectroscopy was used in both natural systems (carotenoids) and artificial systems (carotene-porphyrin-fullerene triad). Next, some new probing techniques and newer light sources were introduced that combine electric and vibrational spectroscopy, higher order spectroscopy, and faster, yet broader, spectroscopy techniques (frequency-comb) are starting to emerge. Researchers have also begun to look at entangled photon spectroscopy, which have been used to provide higher resolution due to the higher selectivity and reduced background noise.

Some of the challenges in this field involve differentiating between coherence and true quantum effects, and distinguishing the electronic, vibrational and vibronic contributions. There is a need and an opportunity to understand the role of coherence in enhancing functionality (sensitivity or selectivity) in a system. Moreover, finding ways to generate entangled photons with sufficient intensity remains a challenge.

A question was asked about the sufficient amount of intensity of entangled photon that is needed. Konar stated that the number should be such that the signal to noise ration should be at least 30 percent.

Girish Agarwal (Texas A&M University) talked about some of the results of smart sensing with squeezed light. Squeezed light are entangled photon pairs that rely on various detection schemes for smart sensing. He described the difference between coherent and squeezed light, where coherent light has quantum fluctuations with uniform distribution in both amplitude and phase. Squeezed light is such that one reduces either the fluctuation in amplitude or phase, and the remaining component is considered squeezed. The squeezed light is of considerable importance in metrology and for improving sensitivity.

Agarwal then described the production of squeezed light using four-wave mixing in atomic Rubidium vapor, and looking at the intensity – the difference squeezing at higher/lower frequency (e.g, ±3 GHz). They have been able to typically generate 6.5 dB intensity difference squeezing.

This squeezed light has been used for entangled two-photon microscopy in biomedical imaging. A key aspect is that the quantum correlated photon pairs can vastly enhance the absorption process, which depends linearly rather than quadratically on the photon-flux density. As a demonstration of the improvement in using squeezed light, Agarwal showed a doubling of the fluorescence signal of 4-dicyanomethylene-2-methyl-6-*p*-dimethylaminostyryl-4 H-pyran (DCM) for squeezed light vs coherent light at the same laser intensity. In using a more standard fluorophore, fluorescein, used in biology, the same quantum light generated the same fluorescence signal at much lower power and thus less likely to damage the biosample. Future work would be to test/optimize for other common fluorescent biomarkers and to use this light for other types of non-liner processing, such as upconversion, for sensing.

Roman Kostecki (University of Adelaide) described how biophotonics can be used to probe the body, specifically creating microstructured optical fibers for enhanced sensing. As an example, he described the deliberate introduction of air-holes into a core fiber for the purpose of containing the sample chamber, as well as better control of the guided light with the sample. With functionalized molecules attached to these optical fibers, one can detect the analyte of interest, whose fluorescence signature is a function of the concentration, which can be monitored dynamically over time.

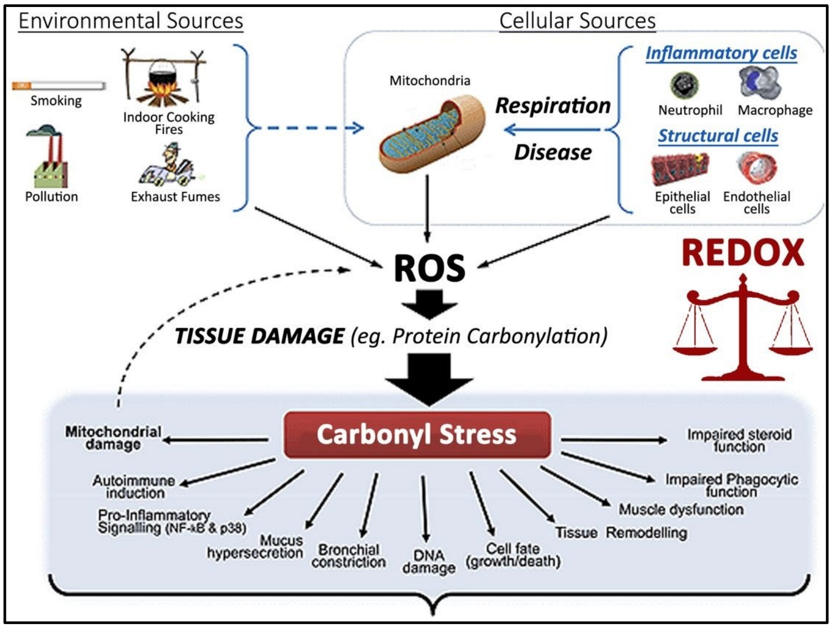


Fig. 9 Using redox changes for a foundation in environmental sensing.

Kostecki described a biological process where a plant leaf would send a signal to other parts of the plants that would alert the rest of the plant for an anticipatory defense response to threats such as caterpillars that eat the leaves. This phenomenon can be used in a variety of applications where one is monitoring the oxidative stress as a biochemical process. Environmental factors such as pollution, create a cellular response that can manifest itself in the form of carbonyl stress. So, by having a dynamic measurement of protein, carbonylation can provide a means for detecting between a healthy and unhealthy state.

Vladislav Yakovlev (Texas A&M University) discussed quantum sensing of classical biophysics and classical sensing of quantum biophysics for the purpose of understanding the basic mechanism over multiple length scales. In these areas, the funding support is dominated by the Chinese government.

Given that nature has been continuously engineering for more than 3.5 billion years, it is hard to imagine that it hasn't made cleaver use of quantum effects. Yakovlev described how quantum sensing can make an impact to either: 1) provide an answer to an open question in biology, 2) provide a new methodology in diagnosing a disease, and 3) provide a new way to treat a disease. He goes on to describe classical probing techniques that incorporate quantum phenomenon, such as Förster resonance energy transfer (FRET), to advance biology. Other areas of interest include leveraging electric fields (Stark effect) and applying it to study electrical potential measurements of cellular membranes. As a specific example, he described applying these techniques to studying microtubules and its dynamics under the influence of a high frequency (GHz) electric field. If such an effect exists, does this influence cellular processes at a higher length scale (e.g., cell-cell communication). After exposure to the GHz electric field, the vibrational modes of the microtubules are modified in two distinct regions (low vibrational frequency (500 cm^{-1}) and higher vibrational frequency (3500 cm^{-1}). Moreover, the water structure, also seen to be modified as a result of the exposure to the GHz electric field. Yakovlev next talked about using quantum light (entangled two photon absorption) to conduct hyper Raman spectroscopy to distinguish (detect) chiral molecules more easily compared to other techniques (Fig. 10.).

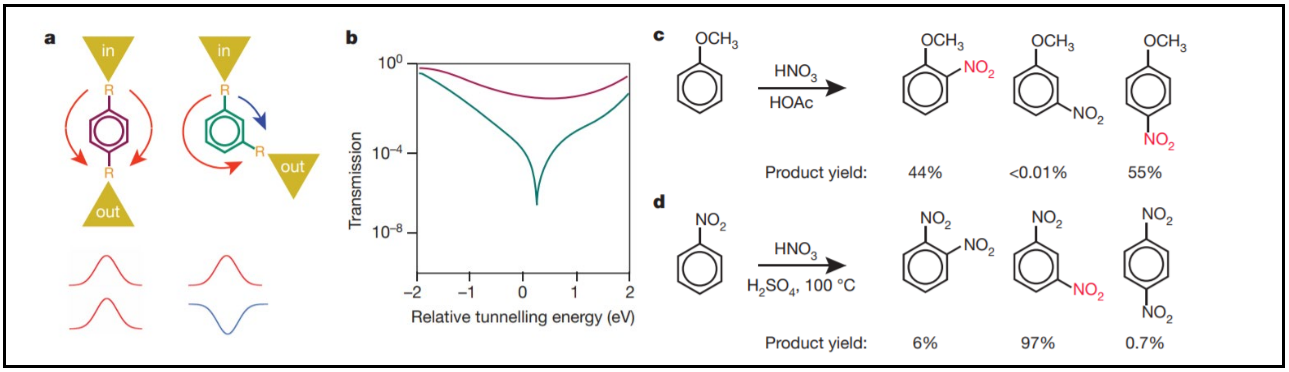


Fig. 10. Theoretical calculation of variation in relative tunneling transmission as a result of different positional isomer in a molecule.

6.0 Panel Session: Non-Classical Probes Monitoring Living Systems

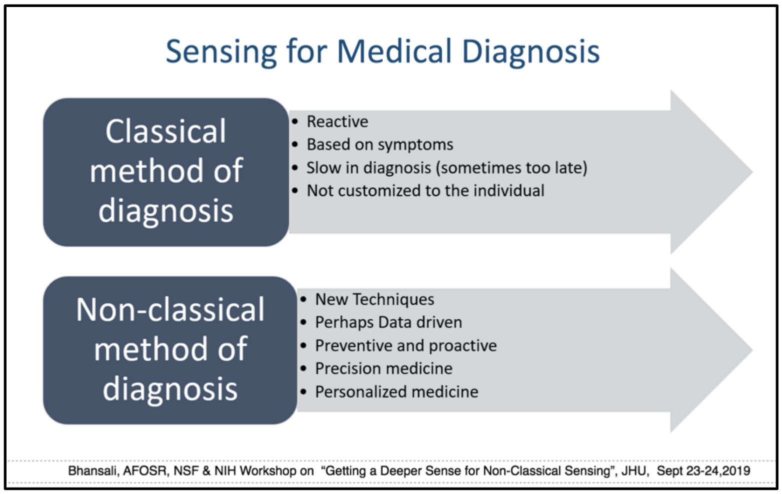
This session on **Non-Classical Probes Monitoring Living Systems**, moderated by **Holly Goodson** of the University of Notre Dame, consists of the of the following key take home messages:

- In health-related sensing applications, we need sensors that can be predictive and not just reactive. In other words, it would be good to be able to detect the onset of a disease, not simply report once symptoms have become obvious;
- Challenges to meeting the goal of developing predictive sensing devices include (a) that such sensors would likely need to be wearable and would thus need to be much more robust than is presently practical; (b) that entities (e.g., government, employers, insurance companies) might try to misuse these data;
- The advent of such a sensor would likely require AI to identify relevant parameters and/or groups of parameters;

- The need for improved health-related sensors is especially acute for animal research, even more so for research involving animals in BSL3 and BLS4 facilities. The need for improved sensors here ranges from simple parameters, such as temperature, to more complex assessments, such as animal stress/suffering and appropriate endpoints (i.e., when it is time to sacrifice an animal that has been given a disease as part of a therapy trial).

The first speaker of this session was **Shekhar Bhansali** (Florida International University). Bhansali's Ph.D. training was in electrical engineering, and his expertise is in areas including bioMEMS, microsensors, nanosystems, and nanomanufacturing. His presentation focused on the problem of developing improved sensors for assessing human health. He started his discussion by pointing out that traditional medical sensing is reactive (it is employed only after disease has occurred) and generic (the same small set of sensors are used in medicine regardless of the disease, person, or body part). Improved sensing (*i.e.,* "smart sensing") would be able to detect disease before it reaches the stage of being symptomatic (Fig. 11). However, there are many challenges to meeting the goal of developing such sensors for use in humans. One is the environment. For example, wearable sensors need to be able to deal not only with body temperature and humidity but the conditions of summer in Miami (or winter in the Midwest). A second and even more daunting problem is that as yet we know too little about how the body works in health and sickness to know what to try to sense. One approach being used to circumvent this lack of knowledge is artificial intelligence (AI). While this technology is powerful, it too has problems. One concern with AI is establishing the data you collect, and how you collect it. Doctors in different places and/or with different training can use different terminology and approaches, which can interfere in a serious way. Problems created by human input lead back to the utility of sensors in medicine, since they can be made to provide standardized input to the AI. A second problem with AI in medicine is that it can be biased. For example, it would not be surprising for a large group of doctors (e.g., dermatologists) to use the same AI provider. If that provider were influenced by a drug company to suggest their drug for a particular condition, the use of AI would be biasing medicine. An additional concern about the use of sensors is that the data they produce creates concerns about privacy and misuse of data.

Overall, the use of sensors and AI to interpret the data has many advantages. These include increased speed, accuracy and precision for the patient, and decreased workload for the health care providers. Disadvantages include potential loss of jobs, as well as decreased human interaction between patient and doctor. Regardless of the concerns, the potential for applications of smart sensors in medicine are enormous because they should help personalize medicine.



Sensing for Medical Diagnosis

Classical method of diagnosis

- Reactive
- Based on symptoms
- Slow in diagnosis (sometimes too late)
- Not customized to the individual

Non-classical method of diagnosis

- New Techniques
- Perhaps Data driven
- Preventive and proactive
- Precision medicine
- Personalized medicine

Bhansali, AFOSR, NSF & NIH Workshop on "Getting a Deeper Sense for Non-Classical Sensing", JHU, Sept 23-24,2019

Fig. 11. *Non-classical sensing has the potential for proactive diagnosis in medicine.*

Question from the audience: How do you define classical and non-classical sensing? Bhensali's answer: "Classical sensing in my mind is biochemistry, imaging, genomics, proteomics, anything that is the standard of care. Non-classical sensing includes the use of artificial intelligence and machine learning, and is when sensors have new modalities (e.g. quantum sensors) or when they are used in a new way."

The next speaker was **Seshadri Vasan**, a public health expert and Rhodes Scholar who leads the Commonwealth Scientific and Industrial Research Organization Dangerous Pathogens team at the Australian Animal Health Laboratory (now called the Australian Center for Disease Preparedness, or ACDP). This is a large biosafety level 3 and 4 facility that works to protect people, livestock, and aquatic organisms from emerging infectious disease threats. He described this facility as "a box in a box in a box in a box in a box in a box in a box" that has cascaded negative pressure zones and a suite of impressive protective measures to ensure that no organisms escape.

Because a major role of the ACDP is to protect livestock from infectious disease, the ACDP must use these animals in its research and when relevant in the BLS4 part of the laboratory. Working with infected large animals is very stressful, dangerous, and demanding work. Thus, development of better remote sensors to assess the physiology of the animals would greatly assist their work. Even improving something as simple as measurement of temperature would be helpful.

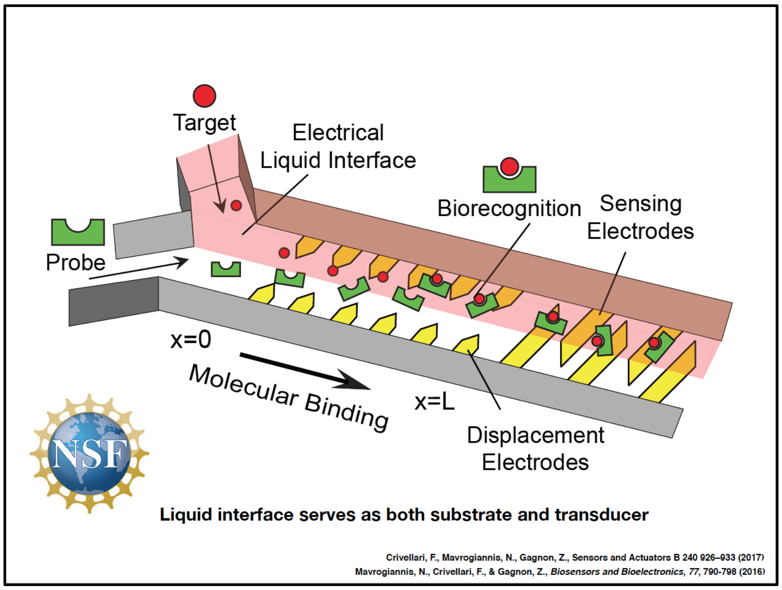
Better and more complete assessment of the animal physiology is important not just to assess disease progression, but to determine humane endpoints, (*i.e.,* to determine when an animal has become so sick and is undergoing so much suffering that it must be euthanized). This issue is extremely important because to test therapies for lethal pathogens, one first needs to make animals sick and then give the therapy a chance to work. Having improved sensors to help determine whether such an endpoint has been reached, would greatly assist their efforts, which are essential to protect livestock and humans from emerging pathogens.

Dr. Vasan finished his presentation by reiterating enthusiasm for collaboration to assist in developing better remote sensors for assessing the physiology of the animals. He did remind potential collaborators that if a sensor goes into his P4 facility, it does not come out. He explained that such collaboration would be on a no-cost basis, and pointed out that testing in his animals could benefit both groups by being a useful step towards eventual testing in humans.

Zachery Gagnon (Texas A&M) gave a talk in this session that was moved from the "Essential Ingredients" session. His talk had several take-home messages:

- Every sensor, classical or non-classical, has three elements: a recognition event, a way to transduce this event, and a way to detect it. To develop non-classical sensors, we need to look at new ways to develop or combine these elements;
- Most recognition events in classical sensing occur at surfaces: one way to develop non-classical sensing is to move into the fluid;
- A major challenge for development of non-classical sensing is to convince industry to manufacture these unusual ("weird-looking") devices.

Dr. Gagnon's talk focused on electrokinetic transducers, and specifically on dielectrophoresis, a process in which one drives phenomena with alternating electric currents (Fig. 12). This method has a number of advantages, including that it is label free. It can incorporate information from multiple parts of the cell (*i.e.,* it is "multi-domain": membrane, cytoplasm, cell wall), and it has very small sample requirements (microliter). However, it also has some disadvantages: it produces an inherently average measurement; it is low throughput, and it lacks specificity. However, this combination of attributes means that it can be used for applications such as determining whether athletes have been giving themselves blood transfusions. Dr. Gagnon finished his talk by discussing how the dielectrophoresis idea can be combined with biological recognition elements to generate a specific sensor where the recognition can be performed in fluid (not on a surface as in other sensors) and that performs at high sensitivity. A major challenge for development of this type of sensor is convincing industry to produce it.



Liquid interface serves as both substrate and transducer

Crivellari, F., Mavrogiannis, N., Gagnon, Z., Sensors and Actuators B 240 926–933 (2017)
Mavrogiannis, N., Crivellari, F., & Gagnon, Z., *Biosensors and Bioelectronics, 77*, 790-798 (2016)

Fig. 12. Advances in sensing can be achieved by designing platforms in which the sensing interactions occur in liquid instead of on hard surfaces.

7.0 Panel Session: Essential Ingredients

This session on **Essential Ingredients**, moderated by **Chenzhong Li** of the National Science Foundation, consists of the of the following key ideas:

- To make progress in identifying and harnessing non-classical mechanisms for sensing, we need collaborations between theorists and experimentalists, and across disciplines;
- One key type of non-classical phenomena that is harnessed frequently by biology for sensing tasks is collective behavior. These collective behaviors can have many different manifestations and are seen at scales from collections of molecules to collections of cells to collections of organisms;
- Collective behaviors can allow sensing of aspects of the environment at one scale (e.g., molecular) to engender responses at larger scales (e.g., whole cell or whole organism);
- A related idea is that sensing power can be improved through multiplexing;
- An interesting question that arises from these points is how collective behavior differs from multiplexing. Multiplexing could perhaps be described as when a system reports on the outcome of many independent devices/tests. In multiplexing, the response is additive. Collective behavior is when the outcome depends on interaction between otherwise independent entities/devices. In this case, the response can be multiplicative and/or exponential. Both can be

very powerful. In designing sensing systems, it might be worthwhile to consider how a multiplexed system could be converted into a system that exhibits collective behavior.

The first speaker of this session was **Moumita Das** (Rochester Institute of Technology). Das's research focuses on collective behavior in soft materials. She is a theorist who works closely with experimentalists, and she is particularly interested in studying the emergence of collective behaviors in multi-scale systems in which there is an interplay between mechanics, statistical mechanics, geometry, and structural properties.

Das gave a very interesting presentation in which she took the session title to heart and identified a number of important "key ingredients" for non-classical sensing. In so doing, she stressed a number of central ideas that connect to other sessions throughout the workshop. These key ingredients included the following:

- Collaborations between theorists and experimentalists and across disciplines are essential to making progress in this field;
- Das stressed the one key common feature to non-classical sensing as seen in biological systems: it involves collective behaviors. Indeed, the cell itself represents collective behavior;
- These collective behaviors allow cellular responses to occur at a length scale larger than the aspect of the environment being sensed. They also allow the response to be non-local. For example, cells can detect molecules and, by engaging the cytoskeleton, move towards the source;
- These collective behaviors can be both equilibrium and non-equilibrium. When the collective behavior is non-equilibrium, the harnessing of energy can improve the signal transduction process;
- These collective behaviors frequently involve phase transitions. The proximity of biological sensing systems to phase transitions allows sensitive responses to small stimuli.

The next speaker was **Sapna Deo** (University of Miami). Deo is Professor of Biochemistry and Molecular Biology at the University of Miami. Her biotechnology lab focuses on developing tools based on luminescent proteins and inorganic probes for the goal of developing rapid, portable, inexpensive, paper fluidic-based, and instrument-free methods for analytical tasks including pathogen detection.

Deo’s talk covered some material similar to that of Das, but additional important points included the following. First, in common with some other talks, she discussed the term “biosensor,” which can be used to describe a device (biologically based or not) that senses a biologically relevant part of the environment; alternatively, a biosensor can be a type of sensor that uses biological components to sense a species of interest. She pointed out that at present, we may be considering too small a list of possible signals and reporters, and that biology can provide additional inspiration and ideas. One additional response modality that deserves more consideration is molecular switches (*e.g.,* calmodulin, intrinsically disordered proteins that assume a structure when binding to the analyte). These molecular switches can be useful by themselves but become even more powerful when combined with reporters such as fluorescent molecules.

The third speaker of the session was **Sun Jin Kim**, (University of Miami). He is also Director of the BioNIUM nanofabrication facility. Kim’s lab focuses on development of nanophotonics for biomedical applications, as well as nanostructured devices for energy capture and sensing devices.

Kim’s talk focused on sensing applications of a phenomenon called Localized Surface Plasmon Resonance (LSPR), which can be harnessed when using appropriate nanoparticles to detect in a very sensitive and bio-compatible way, environmental changes. Classical LSPR utilizes color changes of the nanoparticles. In this talk, he focused on the efforts of his lab to develop a different read-out for the LSPR effect. More specifically, he showed how they are developing a device called a Plasmon Field Effect Transistors (FET), in which the LSPR effect is used to directly generate an electric signal. This device has a sensitivity 5x improved over classical LSPR, and it is compatible with colored matrices (e.g., blood). An interesting question here is how this is non-classical. SPR/LSPR itself could potentially be considered non-classical, but another aspect of this talk that fit in with the non-classical sensing theme is that Kim stressed the importance of combining different sensing modalities, as well as the power of multiplexing these sensors.

Emanuela Saracino, a Ph.D. student in the Benfenati lab at the National Research Council of Italy (CNR) in the Institute of Organic Synthesis and Photoreactivity (ISOF) shared her research on the development of new tools to sense and modulate astrocytes, the non-neuronal cells in the brain. She shared how her research was enhanced with the help of international collaboration with Wolfgang Losert’s lab and how that collaboration was strengthened by the participation of her lab in a previous workshop on non-classical sensing. This collaboration gave her the opportunity to travel multiple times to the University of Maryland at College Park to collect and share data, and she believes the collaboration will continue to yield great results. She then discussed her work looking at the impact of actin on channel protein dynamics in astrocytes by using gold nanoclusters, which was done in collaboration with Dr. Shashi Karna, U.S. Army Research Laboratory. She concluded by discussing how she plans to connect our understanding of quantum biology, astrocytes bioenergetics, and reactive oxygen species (ROS) to better understand what happens in the brain, and by thanking the community of researchers interested in non-classical sensing for their work in establishing the field.

Kate O'Neill, a postdoctoral fellow in Wolfgang Losert's lab at the University of Maryland, finished the session by discussing her side of the collaboration between the Losert and Benfenati labs and some of her work studying the intracellular dynamics of neural cells. In her work, she studies both the electrical activity in the brain, which occurs on a msec timescale, and also the cytoskeletal dynamics of neural cells, which can occur across a wide range of length scales (from mm to less than an mm for actin, their model of the cytoskeleton). The lab cultures neurons *in vitro*, which are capable of forming networks due to the dynamics of the cytoskeleton that change over the lifetime of the neuron. She then discussed her collaborative work with the Benfenati lab, where they extended their work to study astrocytes. They studied how the cytoskeleton of astrocytes is affected by changes in the extracellular environment, such as alterations to the water or salt concentrations in the media, and how these changes may be related to astrocyte's homeostatic function in the brain.

8.0 Wrap-up & Next Steps

Larry Nagahara (Moderator): This workshop began with an example of outside the box thinking of applying non-classical sensing. Upconversion nanoparticles (UCNPs) are a class of optical transducers in which shorter-wavelength emission occurs via the conversion of long-wavelength excitation that exists in long-lived intermediate energy states of lanthanide ions. One potential application of UCNPs is using them in live cell imaging as optical probes/markers similar to quantum dots. In a recent Cell paper, Ma and co-workers applied UCNPs for a novel kind of non-classical sensing, namely, anchoring UCNPs on retinal photoreceptors to allow for 'seeing' light over 700 nm in wavelength. Based on a series of recordings and both visual behavioral tests, the authors demonstrated that mice with these UCNPs in their retina could not only perceive NIR light, but also see NIR light patterns. The example is to highlight how a community of disparate perspectives, such as those gathered here at this workshop, could come together and think how non-classical sensing can emerge as a new field of study and what steps are needed to take this field to the next level.

Mike Espey carried on the conversation from an agency perspective. There are a lot of awards that are made to outstanding investigators doing individual projects. Dr. Espey felt that the individual projects need to coalesce so that investigators are not in isolation and can bring a system level of understanding, eventually taking the best ideas, and integrating them. You would want to integrate them in a complimentary way such that the whole is greater than the sum of their parts. This integrated system could be used for diagnostics, monitoring its surroundings, determining 'healthy' vs. 'sick', etc. Can we take a suite of orthogonal systems that would have a specific context of us? One way to help integrate is to develop a 'standard' or common model/platform in which everyone could test their concept. Here, having engineered biological systems (i.e., tissue engineering) could play a key role. The suite of orthogonal sensing modality does not have to be completely novel, a combination of both 'classical' and 'non-classical' takes advantage of the best in both worlds and should be encouraged as well. An example is how the body uses various sensory nerve cells and that information is fed to the vegas nervous system and processed by the brain. Returning to an agency perspective, integrating activities with various U.S. and international agencies (have a collective behavior approach) will also help catalyze the nascent field of non-classical sensing (as an emergent new property).

Holly Goodson reminded the audience of two cross cutting themes that emerged from this workshop, namely, sensors for biological molecules and biology that does sensing. She encouraged the engineers, who are designing sensors to study biological molecules, to think about how to harness "the biology" where possible as biological systems have been honed for hundreds of millions to billions of years. While these biological systems are stunning, we can also augment them, as with the example that Dr. Nagahara just presented by allowing living systems (mice) to see in the IR.

Wolfgang Losert discussed the large volume of data that could potentially be generated in this effort, and the steps that should be taken as a community to share this data so that one laboratory's results could be useful to other laboratories and vice versa. This group could overcome this challenge as we have become more familiar with each other over the past couple of days. To reiterate the theme of sensors for biological molecules and biology that does sensing, one could also add actuators to this group as well.

Participants Discussion was expressed in the following summaries and suggestions for going forward. While biosensing is important, another common theme in this workshop has been the need to both understand and control non-classical behavior/functionality across multi-scales (spatial and temporal) through monitoring. There is an equal need for more 'ex-vivo' research to complement the ongoing in-vivo/in-vitro activities. Having information at the cell, organ, or tissue-level on different properties, whether physical, chemical, electrical/magnetic, in real-time is needed for this field.

The large variety of perspectives in this workshop made it truly unique, especially having trainees, along with senior and junior investigators, present their research and providing feedback on this field was refreshing. The ex-vivo discussion is important in that there is a system where you can test things. Ideally, this will allow for global capacity to test and share the information and more importantly, validate the data. Testing something in South Africa, Australia, Italy, or the U.S. on an ex-vivo system and having a common dataset will rapidly move the field forward. Another key value of this workshop is the potential for innovation, where a specific application is not necessarily the point of discussion or emphasis but acts as a seed.

The tools that ultimately come out of this workshop should not be thought of merely as building a new class of biosensors for detection use only, but also for examining fundamental properties of a cell or microenvironment of tissues. This class of non-classical sensors can have an impact on monitoring the structures or understand new biological function. For example, an organ on a chip (tissue engineering) needs biosensors to advance this field. Lastly, this goes back to the dualism and interplay of having sensors to improve/optimize biology, which in turn, uses the optimized biology as a sensor itself, such as bio-dosimetry.

Acknowledgments

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Appendix

1) **Participant List**

2) **Workshop Agenda**

JOHNS HOPKINS
WHITING SCHOOL
of ENGINEERING

in collaboration with

NIH NATIONAL CANCER INSTITUTE

WORKSHOP ON
"GETTING A DEEPER SENSE FOR NON-CLASSICAL SENSING"

PARTICIPATION LIST

Girish Agarwal
Texas A&M University
girish.agarwal@tamu.edu

Valentina Benfenati
Institute of Organic Synthesis & Photoreactivity
National Research Council of Italy
valentina.benfenati@isof.cnr.it

Sofi Bin-Salamon
Air Force Office of Scientific Research
sofi.bin-salamon@us.af.mil

Shekhar Bhansali
Florida International University
sbhansa@fiu.edu

Paul Brumer
University of Toronto
pbrumer@chem.utoronto.ca

Jeff Buchsbaum
National Cancer Institute
National Institutes of Health
jeff.buchsbaum@nih.gov

Giulio Busulini
George Washington University
giuliobusulini@gwu.edu

Franklin Carrero-Martinez
National Academy of Sciences
FCarrero@nas.edu

Yun Chen
Johns Hopkins University
yun.chen@jhu.edu

Bianxiao Cui
Stanford University
bcui@stanford.edu

Moumita Das
Rochester Institute of Technoloty
modsps@rit.edu

Sylvia Daunert
University of Miami
sdaunert@med.miami.edu

Sapna Deo
University of Miami
sdeo@med.miami.edu

George (Yegor) Dubynskyi
Embassy of Ukraine
georgii.dubynskyi@mfa.gov.ua

Michael Espey
National Cancer Institute
National Institutes of Health
michael.espey@nih.gov

Nicole Forrester
Commonwealth Scientific and Industrial Research Organisation
nicole.forrester@csiro.au

Zachary (Zach) Gagnon
Texas A&M University
zgagnon@tamu.edu

Sharon Gerecht
Johns Hopkins University
gerecht@jhu.edu

Holly Goodson
University of Notre Dame
holly.v.goodson.1@nd.edu

Henry Hess
Columbia University
hh2374@columbia.edu

Shashi Karna
Army Research Laboratory
shashi.p.karna.civ@mail.mil

Sung Jin Kim
University of Miami
kim@miami.edu

Arkaprabha (Arka) Konar
Kent State University
akonar@kent.edu

Roman Kostecki
University of Adelaide
roman.kostecki@adelaide.edu.au

Chenzhong Li
National Science Foundation
chli@nsf.gov

Wolfgang Losert
University of Maryland, College Park
wlosert@umd.edu

Misoon Mah
Air Force Office of Scientific Research
misoon.mah@us.af.mil

Carlos Martino
Florida Institute of Technology
cmartino@fit.edu

Read Montague
Virginia Tech University
read@vtc.vt.edu

Kristopher (Kris) Murray
University of Notre Dame
kmurray5@nd.edu

Larry Nagahara
Johns Hopkins University
larry.nagahara@jhu.edu

Kate O'Neill
University of Maryland, College Park
oneill.katem@umd.edu

Zhijian Pei
Texas A&M University
zjpei@tamu.edu

Shashank Priya
Pennsylvania State University
sup103@psu.edu

Emanuela Saracino
Institute of Organic Synthesis & Photoreactivity
National Research Council of Italy
emanuela.saracino@isof.cnr.it

Yuko Tsuda
Japan Science and Technology Agency
yuko.tsuda@jst.go.jp

Robert Usselman
Florida Institute of Technology
russelman@fit.edu

Seshadri (Vasan) Vasan
Commonwealth Scientific and Industrial Research Organisation
University of York
Vasan.Vasan@csiro.au

Vladislav (Vlad) Yakovlev
Texas A&M University
yakovlev@tamu.edu

Kaiming Ye
Binghamton University
kye@binghamton.edu

Theodoros (Theo) Zanos
Feinstein Institute for Medical Research
Hofstra Northwell School of Medicine
tzanos@northwell.edu

Scribes:

Kristopher (Kris) Murray
University of Notre Dame

Kate O'Neill
University of Maryland, College Park

Conference Support:

Michelle Cagan
Johns Hopkins University

Jennean Everett
Johns Hopkins University

Linda McLean
Johns Hopkins University

NSF

JOHNS HOPKINS
WHITING SCHOOL
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NIH NATIONAL CANCER INSTITUTE

WORKSHOP ON
"GETTING A DEEPER SENSE FOR NON-CLASSICAL SENSING"

September 23-24, 2019

School for Advanced International Studies (SAIS)
Johns Hopkins University
(1740 Massachusetts Ave. NW, Washington DC)

Kenney-Herter Auditorium

MEETING AGENDA: Day 1 (Monday, September 23rd)

8:00 a.m. – 8:30 a.m. **Registration & Continental Breakfast**

8:30 a.m. – 8:35 a.m. **Welcome & Introductions**

Organizers:

Larry Nagahara
Johns Hopkins University

Michael Espey
National Cancer Institute/National Institutes of Health

Holly Goodson
University of Notre Dame

Wolfgang Losert
University of Maryland, College Park

8:35 a.m. – 8:45 a.m. **Workshop Ground Rules & Recap: Non-Classical Behaviors in Biological Functions: Potential for Smart Sensing Workshop**

8:45 a.m. – 12:00 p.m **Session 1: Overarching Principles of Non-classical Behaviors**

8:45 a.m. – 10:20 a.m. **Vignette #1: "Dosimetry" & Biological Function**

Moderator: Michael Espey
National Cancer Institute/National Institutes of Health

Panelists: Valentina Benfenati
The Institute of Organic Synthesis and Photoreactivity
National Research Council of Italy

Jeffrey Buchsbaum
National Cancer Institute/National Institutes of Health

Bianxiao Cui
Stanford University

Read Montague
Virginia Tech University

Theodoros (Theo) Zanos
Feinstein Institutes for Medical Research &
Hofstra Northwell School of Medicine

10:20 a.m. – 10:40 a.m. **Break**

10:40 a.m. – 12:00 p.m. **Vignette #2: Collective Behavior**

Moderator: Holly Goodson
University of Notre Dame

Panelists: Sylvia Daunert
University of Miami

Holly Goodson
University of Notre Dame

Wolfgang Losert
University of Maryland, College Park

Shashank Priya
Pennsylvania State University

12:00 p.m.– 1:30 p.m. **Working Lunch: Potluck – International Research Experiences**

Moderator: Shashank Priya
Pennsylvania State University

Panelists: Giulio Busulini
George Washington University

Franklin Carrero-Martinez
National Academy of Sciences

Nicole Forrester
Commonwealth Scientific and Industrial Research Organization

Misoon Mah
Air Force Office of Scientific Research

Yuko Tsuda
Japan Science and Technology Agency

1:30 p.m. – 2:10 p.m. **Panel Session 2: Manufacturing on Smart Sensors**

Moderator: Larry Nagahara
Johns Hopkins University

Panelists: Zhijian Pei
Texas A&M University

Kaiming Ye
Binghamton University

2:10 p.m. – 3:10 p.m. **Session 1 (Continued): Overarching Principles of Non-classical Behaviors**

2:10 p.m. – 3:20 p.m. **Vignette #3: Quantum Behavior in Biological Functions**

Moderator: Wolfgang Losert
University of Maryland, College Park

Panelists: Paul Brumer
University of Toronto

Henry Hess
Columbia University

Carlos Martino
Florida Institute of Technology

Robert Usselman
Florida Institute of Technology

3:20 p.m. – 3:40 p.m. **Break**

3:34 p.m. – 4:50 p.m. **Panel Session 3: Optical Probing Non-Classical Phenomena**

Moderator: Michael Espey
National Cancer Institute/National Institutes of Health

Panelists: Arkaprabha (Arka) Konar
Kent State University

Girish Agarwal
Texas A&M University

Roman Kostecki
University of Adelaide

Vladislav Yakovlev
Texas A&M University

4:50 p.m. – 5:00 p.m. **Wrap-up for Day 1**

5:00 p.m. **Adjourn**

MEETING AGENDA: Day 2 (Tuesday, September 24th)

8:30 a.m. – 9:00 a.m. **Registration & Continental Breakfast**

9:00 a.m. – 9:10 a.m. **Summary of Day 1**

Organizers:
Larry Nagahara
Johns Hopkins University

Michael Espey
National Cancer Institute/National Institutes of Health

Holly Goodson
University of Notre Dame

Wolfgang Losert
University of Maryland, College Park

9:10 a.m. – 10:20 a.m. **Panel Session 4: Non-Classical Probes Monitoring Living Systems**

Moderator:
Holly Goodson
University of Notre Dame

Panelists:
Shekhar Bhansali
Florida International University

Zachery Gagnon
Texas A&M University

Seshadri (Vasan) Vasan
Commonwealth Scientific and Industrial Research Organization
University of York

10:20 a.m. – 10:50 a.m. **Break**

10:50 a.m. – 12:00 p.m. **Panel Session 5: Essential Ingredients**

Moderator:
Chenzhong Li
National Science Foundation

Panelists:	Moumita Das Rochester Institute of Technology
	Sapna Deo University of Miami
	Sung Jin Kim University of Miami
	Kate O'Neill University of Maryland, College Park
	Emanuela (Manuela) Saracino Institute of Organic Synthesis & Photoreactivity National Research Council of Italy

12:00 p.m.– 1:00 p.m. **Working Lunch: Wrap-up, Next Steps, and Adjourn**