Report on the Non-Classical Behaviors in Biological Functions: Potential for Smart Sensing Workshop

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Summary:

The development of novel devices, structures, and materials through bio-inspiration offer the potential for orthogonal approaches in monitoring, processing, and controlling mechanisms in a wide range of complex environments. An important and emerging subset in this field is non-classical behaviors occurring in biological systems and its potential application toward smart sensing. To determine the current status, research gaps, and necessary steps to advance the field, a two-day strategic workshop was held to explore various types of non-classical behavior, such as quantum phenomena, biophysical processes, and collective behavior, in the context of biological systems. Moreover, the workshop explored how these non-classical behaviors could be exploited as a probe and whether such a probe could be used to interrogate biological systems. Some of the opportunities discussed included development of sensors utilizing non-classical phenomena as well as invoking the broader international community to help catalyze this nascent multidisciplinary field in both fundamental understanding as well as accelerating innovative applications.

1.0 Introduction

In recent years, there has been increasing evidence of non-classical (*e.g.,* quantum mechanical, biophysical processes, collective behavior, etc.) behavior appearing to play non-trivial roles in certain biological function. These examples appear wide-ranging from photosynthesis being ubiquitous in plants to magnetoreception or collective motion in certain animal species. Just as nature may leverage quantum effects 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? There has been a growing body of published literature as well as an increasing number of conferences on the fundamental aspects of quantum effects in biology. In contrast, there have been few discussions on the prospects of incorporating quantum biology behavior (or other non-classical behavior) into engineered device structure and systems. Such non-classical biophysical processes can lay the foundation for a new revolutionary class of sensing modalities and opens the possibility of "seeing" (and extending) beyond our current detection capabilities in monitoring our surroundings.

Under this basis, a strategic workshop was organized and hosted by Johns Hopkins University (JHU) and in collaboration with the National Institutes of Health (NIH) on April 12-13, 2018 in Arlington, Virginia. The overarching objectives of this workshop were more than to assemble a group of world-class scientific leaders in the non-classical fields and to update each other on their particular research, but rather bring disparate groups together to address the following:

- *Review (briefly) 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 of non-classical behavior appearing in smart sensors.*

Over the two days, the workshop was partitioned into six sessions covering the overarching objections. The range of topics covered in this workshop is in foundational areas relevant to the Physical and Biological Sciences Branch (RTB) of the Air Force Office for Scientific Research (AFOSR). Moreover, leveraging biophysical behaviors could bring new, orthogonal capabilities to future Air Force systems in the form of designing and implementing advanced sensing devices. In other words, further research in this area would generate fundamental knowledge that could be transformational in enabling U.S. Air Force operations to have enhanced sensing and improved, robust situational awareness. Understanding how living (biological) systems utilize quantum behavior at the nanoscale, which is hierarchically expressed in a "macroscopic" function, could inspire future technology in a wide variety of Air Force applications, such as enabling advanced dynamic control using unconventional sensory systems (e.g., magnetoreception for global navigation), thereby provide more versatility and/or performance (e.g., low energy usage).

2.0 Non-Classical Behavior: What Does it Mean?

From a physics-based perspective, non-classical behavior, such as quantum behavior, is exhibited by systems that do not obey the classical laws of nature. Non-classical behavior at the microscopic level, although short-lived, can have profound effects at the macroscopic level, and the large-scale system may not necessarily exhibit any non-classical behavior. This downstream effect is an important outcome that must be considered when assessing the importance of non-classical effects in biological systems. For example, the quantum oscillations during the cis-trans isomerization of retinal is believed to play some role downstream in the efficiency of vision. In the first panel, Andrew Greentree (Royal Melbourne Institute of Technology) introduced the concept of quantum transport and entanglement and illustrated this idea using the example of diamond nitrogen-vacancy (NV) centers, where non-classical behavior is preserved at room temperature. This system is already being used as a smart sensor, and newer systems are being developed to be used as room temperature smart sensors.

A difficulty with defining non-classical behavior is that at its base, the term simply means that the behavior defies convention. In physics, this convention is classical (i.e., non-quantum) physics, but in biology this convention just means current large-scale trends in methodology. As this conference was designed to explore non-classical behavior at the interface between the two fields, this report will use the less specific definition, belonging to biology. Therefore, under this convention, even techniques which utilize classical physics, such as Raman or Brillouin spectroscopy, are biologically non-classical as they utilize intrinsic molecular properties which are not typically exploited in biological research.

Moreover, collective behavior such as those observed in certain animal species (e.g., birds, fishes, insects) is an emergent non-classical behavior that is not observed if the animal species is in isolation or in small numbers. Collective (coordinated) behavior of large groups of similar animals can enhance the transfer of information – "collective intelligence" – across the group, which can significantly enhance sensory capabilities for identifying and gathering food or sensing and evading from predators. In Session 2, Holly Goodson (University of Notre Dame) introduced the idea of collective behavior as an example of non-classical biological functions and illustrated an example with schools of fishes are much more efficient at finding sources of food than an individual fish.

Based on the presentations and ensuing discussions, it is clear that non-classical behavior means using innovative methods for detecting, targeting, and perturbing cellular and sub-cellular behavior. From a biological perspective, it means exploiting properties of cells and their organelles using a method other than conventional light microscopy. Examples of technologies discussed at the workshop are entangled photon spectroscopy, label-free imaging, and nano-sensors, among others. Pushing the boundaries of detection is important because different cells have unique "signatures" that may come in the form of metabolism, and vibrational frequency. From an organizational perspective – as in the case of MINTEK – non-classical behavior means spanning many areas of the research process with the goal of supporting innovation. In summary, biology has a lot to tell us. We just have to figure out how to improve our detection of biological signals to get the most out of our research.

3.0 Panel 1: Non-Classical Behavior Introduction

Girish Agarwal (Texas A&M University), an expert in the field of quantum optics, began the meeting by reviewing system-probe interactions, and brainstorming ways of determining signatures of "quantumness." He briefly discussed how scientists have used classical probes, such as light emitted from solid-state lasers, to investigate large numbers of biophysical systems, and how evidence of non-classical behaviors has been observed. Agarwal explained that quantum probes have emerged recently as a result of enhanced sensitivity in detecting displacements and motions.. He stressed that an ideal, ultrasensitive quantum probe would need to incorporate the features of both classical and quantum probes and be used in a complementary fashion. Furthermore, Agarwal explained that signatures of quantumness, particularly of the output field, can be assessed by counting the photon number distribution, which, when narrower than Poisson distribution, would indicate non-classical behavior of the output field. This property can be observed experimentally by measuring the second moment of the photon distribution. He showed an example of how two or more detectors can be used together, acting as coincidence detectors. Correlated fluctuations in intensity can be tracked using these coincidence detectors along with the detection of photo distribution parity. Carefully designed classical measurements, such as intensity-phase correlations and cross-correlation, can be employed as well, as long as these measurements are performed when working beneath the standard quantum noise limit. He then reviewed different methods for producing quantum probes, with two of the most widely accepted methods being four-wave mixing in atomic vapors and spontaneous parametric downconversion. Agarwal discussed how these correlated photon pairs can be implemented in several different types of interferometers, where one of the photons interacts with the sample and the loss of correlation is measured by interfering with the other photon. Finally, he noted that people are working to push the sensitivity of the interferometers beyond the Heisenberg limit.

Vladislav Yakovlev (Texas A&M University) raised a question about scattering in biological systems, and how useful entangled photons will be for probing these kinds of systems. Agarwal pointed out that visibility of the interference fringes should not diminish by 50 percent or more. Quantum effects can still be resolved if the visibility goes down by 20-30 percent.

Paul Brumer (University of Toronto), a world leader in the field of theoretical chemical physics and coherent control of light-matter interactions, began his presentation by noting that he will focus on the quantum nature of matter, which will complement the previous talk that emphasized the quantum nature of light. According to Brumer, the first and most important step is to differentiate non-classical behaviors from everyday classical behaviors. He beautifully illustrated this point by showing an example of the double-slit experiment,pointing out some major conceptual differences compared to classical systems. The first is that quantum systems are affected by the very measurements made to record system behavior. This is seen when the same measurements repeated several times yield different results. The second difference is that uncertainty in the pairs of variables, such as position and momentum, leads to Heisenberg's uncertainty principle. The third and the most important concept is that energy is quantized for bound systems, and excitation or de-excitation is allowed as integer multiples of the energy "quanta." Additionally, a somewhat hard to grasp and bizarre concept is that the elements of reality are a set of values or properties that do not exist prior to the measurement. Nonlocality and entanglement are examples of these elements.

"So, if everything in nature behaves quantum mechanically, where and when does classical mechanics take over?" Brumer asked. Or, as put by a famous particle physicist, "Where did all the weirdness go?" The answer to this is "Decoherence!"

Brumer stressed that quantum systems are extremely delicate and can be very easily destroyed by interaction with the surrounding environment. Moreover, he believes that we should learn from the animal visual system in order to build smart sensors based on non-classical phenomena since this system has been perfected over millions of years and works efficiently to drive a cis-trans isomerization reaction when a photon is absorbed by the retinal molecule. Recent evidence of long-lived coherences has generated renewed interest in the scientific community, and experiments have tried to measure detection of single photons by the human eye and test the human visual system as a double-slit interferometer. Brumer closed by pointing out the bizarreness of entanglement, where perturbation of one photon in an entangled photon pair – even when separated by several thousand miles – leads to the perturbation of the other photon. This phenomenon can be somewhat explained by the wave-like character of quantum systems.

Agarwal mentioned the Feynman lectures, where Richard Feynman discusses electron interference and matter-wave interference and compared electrons with bullets in a double-slit experiment, to which Brumer replied that the enthusiasm about double-slit experiments using quantum systems comes from Feynman's lectures. Peter Burke (University of California, Irvine) raised a question about the validity of quantum entanglement in biological systems and pointed out that we do not get two entangled action potentials downstream that are initiated by excitation from a pair of entangled photons. Brumer mentioned a recent work published by Steve Boppart at the University of Illinois Urbana-Champaign regarding the issue of trying to see interference effects downstream by changing the relative phase of two-photon excitation. However, this field is relatively nascent and requires significant investigation. Retinal biology, in particular, might be ideally suited for these types of investigations.

Andrew Greentree (Royal Melbourne Institute of Technology), a leader in theoretical physics and quantum optics, was enthusiastic about the idea of bringing together physicists and biologists to brainstorm ways of designing the next generation of sensors. Greentree's research focuses on understanding quantum transport in quantum systems, and he began by asking several fundamental questions, such as how a particle can be moved from one place to another using the laws of quantum mechanics. The goals for using quantum mechanics are to design faster and more robust ways for building devices like quantum computers. Greentree pointed out that when we combine many interesting quantum mechanical systems, the results are several emergent phenomena. The next issue that was discussed is how to take these emergent quantum mechanical systems and design smart sensors from them. The overall goals are that the world is quantum mechanical and that we need to learn from nature and use these principles to design smart sensors. Greentree's work is mostly inspired by the quest for quantum computers, so he often wonders how we can use information optimally. For example, we know that biological systems can accomplish tasks that we cannot reproduce with standard electronics. How have these biological systems evolved to use information and accomplish these tasks? He moved on to explain the spins that make up bits and qubits, and introduced the Bloch sphere representation of spins. He also discussed where and how spins are oriented between discrete states. Spin can point in any direction, and the measurement will only be detected in the basis of the measuring device. If the particle is not in the up or down direction, one will get random answers. Greentree stressed that entangled photons are correlated and have the same quantum state. These photons also share properties, but the properties are unknown until they are measured. He explained the concept of measuring entangled photon states by demonstrating how two different measurements are correlated or not correlated depending on the measurement basis. Each time the basis chosen by two different observers was identical, the results were correlated, whereas, when the basis was different, the results were mathematically uncorrelated.

Greentree explained the use of nitrogen vacancy centers, which is a remarkable system since the quantum mechanical effects are preserved at room temperature. This system is sensitive to small magnetic field shifts and uses diamond, a biologically inert material that can be used to probe quantum effects in biological systems at room temperature.

Jeffrey Gilman (National Institute of Standards and Technology) is an organic chemist by training and has been working on designing smart polymers. Gilman began his talk by emphasizing how polymer composites are the future of smart sensor materials and how these materials hold the potential to completely revolutionize the field. Polymer composites can drastically reduce the price of large scale structures such as cars, aircrafts, bridges, and liquid tanks. Polymers are also lighter and easier to fabricate than traditional materials. He then discussed how fluorescence is used to monitor the properties of polymers and how these properties act on the nano-scale at interfaces. For example, polymers undergo a phase change around the glass transition temperature, and become more flexible. This technique has been used for quite some time since it is sensitive to phase changes. Furthermore, several polymer materials show nano-scale dynamics on a nano-second timescale even after cooling down, as evidenced by neutron scattering experiments. Gilman gave the example of polycarbonate, a bullet-proof material that responds to high strain as a result of dynamic motions. However, these polycarbonate materials are not suited for designing liquid oxygen tanks since nano-scale motions facilitate small molecule transport. Additionally, two-photon lifetime imaging is used by probing fluorophores specifically designed for use in a super resolution microscopy technique called photo-activated localization microscopy (PALM). Gilman discovered that these molecules were activated in the presence of ultraviolet (UV) photons and moisture and, consequently, can be used as efficient water sensors. He also looked at fluorescence lifetime (FL) distributions in cellulose with hydrophobic and hydrophilic cores, thereby gathering information about polymer dynamics. PALM fluorophores are also stress activated and can be used to measure stress in composites.

Valentina Benfenati (Institute of Organic Synthesis and Photoreactivity, National Research Council of Italy) asked whether the information gained from studying stress response in polymers could be applied to understanding biological systems. Gilman mentioned a similar study from Yale University in which this was done. Agarwal wondered if the lifetime is affected by the dielectric properties of the polymer. Gilman answered yes and explained how for water sensing applications, separate dielectric measurements are performed to probe the dynamics of the bound water.

3.1 General Discussion

Larry Nagahara (Johns Hopkins University) openend a group discussion about Brumer's ideas on the human eye. Is the detection of single photons by the human eye important? What would we see if it was not a single photon? Brumer replied that he was commenting on sensitivity versus versatility. Being able to sense photons more quickly will lead to a better ability to detect threats in the environment. Yakovlev commented that the brain is not fast enough to process such information. Brumer then pointed out an example of cell differentiation, where the ratio of cis-isomer to trans-isomer of retinolic acid completely alters the process. This is an example of a long-term effect on a microscopic process. Burke then wanted to discuss what is quantum versus what is classical. Coherences cannot live for nano-seconds since they are very fragile. NV centers maintain quantum purity and are not affected by environment, unlike retinal, which loses *coherence in picoseconds. Prof. Greentree replied that it ultimately depends on the more efficient transduction of energy or information in the first few picoseconds by the entangled states of quantum absorbers. Agarwal made a few comments about single photons, suggesting that they can be used to look at extremely weak scattering objects. Single photons tend to dephase more slowly when compared to entangled photon pairs. Agarwal asked Brumer if our eyes are sensitive to polarization of light, to which Brumer replied that he would have to check. There was a discussion about the function of hairs on the outer surface of ears helping to amplify sound as well as suppress thermal noise. There are theoretical models called bang-bang models where the effects of thermal noise are suppressed by constant hitting and that maybe something similar is happening in our ears. Benfenati asked what happens if we shine entangled photons on a saline solution? Agarwal suggested an interferometry-type measurement where one photon passes through the solution and the other is used to detect changes. A group from Singapore tried to perform a similar experiment with cells in collaboration with Yakovlev, but the project did not receive funding. Biological systems do not care about single photons since they have evolved to detect predators in order to propagate life. Single photon detection is the absolute limit which has been incorporated in the sensing machinery in order to achieve that goal.*

4.0 Panel 2: Non-Classical Biological Function (Magneto-navigation): From Proteins to Cells

In this session on non-classical biological functions, Holly Goodson (University of Notre Dame) introduced the idea of collective behavior as an example of non-classical biological functions. Challenges that organisms can overcome by using a collective approach range in scale from protein- to community-sized. Collective behaviors tend to be useful in biology for locating things in complex environments, detection of signals below a certain noise threshold, and comparing relative levels of a signal.

Bacteria use chemotaxis based on random walks, which allows them to both detect and move down small chemical gradients. This results in a group of bacteria congregating at the maximum chemical signal. Depending on the cell density, bacteria can sense a quorum, change behavior, and secrete enzymes to cooperate when eating. This combination of random walks and quorum sensing allows for simple and robust detection of food sources, and then effective but slow cooperation through chemical diffusion in water.

Goodson referenced work on collective behavior in fish from the lab of Iain Couzin (Max Planck Institute for Ornithology/University of Konstanz). As a group, schools of fish are much more efficient at finding sources of food than individual fish. This behavior can be modeled with only two simple rules: stay close to your neighbor, and move towards a food source. A trend seen in the group, but not the individuals, is that groups can follow gradients of food sources.

Goodson’s final example of collective behavior is the travel of ants across a space and the subsequent reinforcement of paths that lead to food. A group of ants will begin by randomly exploring a space while leaving a chemical trail. Once it locates food, the ant will follow the trail back, thus reinforcing the chemical trail. Subsequent ants will also follow this trail, further reinforcing a single path leading to the food source. Such a searching method is efficient at locating sparse, randomly distributed resources.

All these examples of collective behavior may have future applications in robotics. Coordinated robots that implement similarly simple algorithms may be more useful for locating resources or objects in complex environments than individual robots.

The next speaker, Moumita Das (Rochester Institute of Technology), began by reviewing the criticality underlying biological responses. Criticality is the idea that, in general, the task of separating a signal from the surrounding background noise is complex. To overcome this issue, biological systems can balance themselves on the edge of a phase transition. This, in effect, introduces a criticality to the system, where small changes will produce intense signals. An example of this property is the mechanical response of actin, microtubules, and intermediate filaments in the cell cytoskeleton. Actin fibers are semiflexible but can undergo glass-like transitions at physiological concentrations, depending on the concentrations and types of crosslinkers present, which can alter the transition points. This allows the shear rigidity and compressibility of cells to be finely tuned by controlling the expression levels of different crosslinkers. Similarly, microtubules have a non-linear mechanical stiffness which can be tuned by concentration.

These phase transitions are also relevant to cancerous cells, which undergo a jamming-to-unjamming transition. Increasing the connectedness of the surroundings can result in a transition where cancer cells can more easily move to other places, resulting in metastasis.

Das concluded by raising a question about the function of mitochondrial networks. The networks are important in cell signaling and cell death. She asked, “Are there enhanced bioenergetic capabilities when mitochondria form networks? If so, do the mitochondria achieve it through some sort of increase in quantum tunneling or collective interaction?” Das intuited that another possibility may be enhanced quality control, whereby healthy mitochondria can fuse freely, while dysfunctional mitochondria will be more likely to dissociate and be removed through mitophagy. In conclusion, Das asked, “Do these networks confer some sort of enhanced signaling capabilities?”

In the third presentation, Maria Procopio (Johns Hopkins University) discussed the magnetic compass in birds as an example of a non-classical sensor in biology. In particular, the magneto-receptor is sensitive to microtesla magnetic fields by employing a quantum coupling strategy. Research into the subject began in the 1970’s, where robins shielded by aluminum cones scratched in the direction of the earth’s magnetic field. By rotating the direction of the field, researchers could change the direction in which the birds scratched. Researchers questioned how this could be possible, given that the geomagnetic field is orders of magnitude smaller than that of thermal noise in biological systems.

One such mechanism that could allow for the detection of the earth’s magnetic field involves a radical-pair reaction in a quantum coherent state that is kinetically, but not thermally, driven. This pathway involves modulation of light detection by the cryptochrome in the eyes. A molecule in the cryptochrome interacts with light that forms an inherently unstable radical pair. The radical pair can interchange between the singlet and triplet states, each of which produce their own reaction products. The ratio of singlet and triplet states is sensitive to the magnetic field due to the hyperfine transition, introducing an anisotropy in the system that can be exploited for determining the direction of the local magnetic fields. This spin selectivity requires coherence, and Procopio posed the following questions: Can thermal noise destroy this coherence, and if so what types of signal-to-noise ratio can be obtained? With each cell having an array of receptors, how many cells are required to detect an emergent signal?

In the final presentation of the session, Robert Usselman (Montana State University), introduced the idea that reactive oxygen species (ROS) can exert quantum biological effects. He first began by reviewing the concept of quantum biology as the idea that quantum phenomena can be observed in biological systems at the functional, cellular, or organism level. It has been hypothesized that flavo-molecules that produce ROSs undergo radical-pair reactions that can be influenced by magnetic fields through the Zeeman effect and hyperfine coupling, similar to the coupling suggested in the mechanism involved in the bird magnetoreceptor. This difference in the triplet yield can be measured via the hyperfine region.

Usselman then discussed how his group sought to determine if this difference in yield is important in cellular ROS processes by introducing and changing the orientation of magnetic fields. There were weak but detectable changes in ROS products. He concluded by saying there was indirect evidence linking the biological production of ROSs to quantum coherence. Furthermore, the production of ROS products may influence cellular bioenergetics, thus acting as a bridge between atomic and cellular levels.

4.1 General Discussion

In the discussion, a lingering question was if mitochondrial networks act similarly to the collective behaviors of ants, fish, or bacteria. Have there been observations of coherent optimal signals from singlet-triplet conversions in the cryptochrome? Are rigidity transitions occurring or involved during specific biological processes? What role does thermal noise play in the coherence of radical-pair processes? And finally, can we use biological quantum sensors to enhance manmade quantum sensors?

5.0 Panel 3: Non-Classical Phenomena as a Probe

Jennifer Ogilvie (University of Michigan) began the session by discussing advances in microscopy through non-linear and non-classical effects. She first addressed time-resolved, non-linear spectroscopy, which can be used to examine networks of Lay absorbers and learn about the system Hamiltonian and the system-bath interactions of a quantum biological system. Regarding the latter point, it is important to recognize that classical coherent effects are often misinterpreted as quantum effects, so care must be taken to distinguish between the two. Additionally, electronic-vibrational resonances may stand to greatly enhance the function of time-resolved spectroscopy. Ogilvie then discussed other non-linear microscopy techniques, such as CARS (Coherent Anti-Stokes Raman Spectroscopy), MPEF (Multi-Photon Excitation and Fluorescence), and pump-probe microscopy, which are all used to enhance biological contrast with classical light. However, by using non-classical light, we can improve these techniques further. Squeezed states and entangled photons can excite weak energy states and give an enhanced signal-to-noise ratio by increasing the two-photon absorption cross-section. Multiphoton microscopy and spectroscopy with entangled photons and other forms of non-classical light can also give better resolution of the system Hamiltonian and virtual states. Ogilvie then identified open challenges in this field, such as preserving non-classical characteristics in biological media (e.g., scattering in tissue can destroy entanglement) and preparing non-classical states in a biological system. There is also a question as to whether non-classical excitation light can better distinguish between classical and quantum phenomena and be used to better understand the latter's role in biological signaling processes.

To this last point, the audience raised a question about whether one could tailor an optogenetic probe to be dependent on quantum states of the excitation light, such as spin.

Next, Mabel Coyanis (MINTEK) discussed existing technologies and possible applications based on non-classical probes. As an example of an application currently being explored, she noted that researchers are currently developing single-molecule sequencing technologies based on transverse electron transport. Transverse reading is faster and cheaper compared to current longitudinal reading technologies. Read speed would be dependent on quantum tunneling effects, which have femtosecond transit times, significantly faster than other existing sequencing methods. Challenges, however, include the coupled effects of thermal noise, bandwidth, and sample resistance. Coyanis then addressed another possible application, controlling water and ion transport in nano-channels. Molecular dynamic simulations use electroosmotic flow to simulate water and ions simultaneously, and through such simulations, a competition between the size of a channel and hydrated ion size (which determines the transport behavior) has been noted. Ideally, non-classical technologies would be able to exploit this competition as a "knob" to turn to control said transport behavior. There is also the possibility of visualizing fluctuations in myelin ultrastructure, which would require highly-spatially-resolved probes. Coyanis' final potential application for non-classical probes was in the study of peptide and protein dynamics by employing nuclear magnetic resonance (NMR) spectroscopy with increased contrast using spin states in probes like the nitrogen vacancy center in nano-diamonds. However, molecular motions at ambient temperature suppress many important types of structural information, so this will present a possible challenge with this particular use case.

Brant Gibson (Royal Melbourne Institute of Technology) discussed the aforementioned topic of the nitrogen vacancy center in diamonds. This vacancy center occurs in bulk space and nano-diamonds, the latter of which can be made from milled, detonated, or crushed diamonds. He discussed recent work involving nano-diamonds loaded into HeLa cells, where optically detected magnetic resonance measurements were made. The orientation of the nano-diamonds could also be detected because the resonance has an angular dependence. Currently, no evidence exists that these nano-diamonds can be taken in by endocytosis, so loading them as was done in the previously mentioned HeLa study remains the only option. He then brought up the possibility of using these nano-diamonds for thermometry inside of living cells. Existing studies have used microwave irradiation to change the fluorescent output signal from the excitation of the vacancy center, where they observed changes that occurred at a resolution of approximately .1 K. By doping optical fibers and PCL substrates and composites with nano-diamonds, Gibson noted that one could also make these technologies sensitive to magnetic fields. One can also bind these materials to specific sensors to enhance these sensors with fine spatial control.

The audience wished to know if these nano-diamonds could be used as a lasing material, to which Dr. Gibson responded that work is currently being done in this space.

In the fourth presentation, Philip Hemmer (Texas A&M University) continued discussing the nitrogen vacancy center in nano-diamonds. He focused on using them to improve contrast in magnetic resonance imaging (MRI), an application previously mentioned by Mabel Coyanis. Hemmer stated that, currently, MRI has enough resolution to study single molecule dynamics, but the signal-to-noise ratio is too low. Optical alternatives are needed to improve this situation. The three ingredients that MRI needs to succeed are polarizability, distinguishability, and detectability. The nitrogen-vacancy center may be able to provide these three ingredients, through an 80 percent spin polarization and 30 percent suppressed fluorescence for $M = \pm 1$ spin states. Additionally, the smallest magnet is a single spin, as is the vacancy center, so one can detect local gradients using the nitrogen vacancy magnetic resonance. It may also be possible to probe neighboring "dark" spins (spins not detectable through photoluminescence) via backaction on the nitrogen vacancy center. Hemmer then highlighted a wish-list for future nano-diamond development. Ideally he would like diamonds that are single-digit nano-meters in size with 100 percent spin contrast, no spin/charge traps, available in N or P types, with a minimum of one complex color center per diamond. Some of these can be achieved by using diamondoids to seed tailored nano-diamond growth. Seed growth increases the number of nano-diamonds harvested by three orders of magnitude, and results in little-to-no nitrogen in the final diamonds. Hemmer noted that, in addition to making polymers of nano-diamonds, one could also use this process to make quantum registers by adding another C13 to the nano-diamonds during growth. One future challenge, though, is that the time resolution of these vacancy centers is currently unknown, due to the lack of high-quality statistical measurements.

For the final presentation of this session, Vladislav Yakovlev (Texas A&M University) returned to the idea of employing non-linear and non-classical microscopy and spectroscopy to enhance current techniques. He noted the characteristics of a good sensor, sensitivity and specificity, and argued that quantum behavior – typified by properties such as periodic motion, discrete spectra, and phases – stands to increase those two characteristics in existing sensors. He discussed current limitations in Raman spectroscopy, namely its specificity. Because other types of spectroscopy, such as Zeeman or Stark spectroscopy, are sensitive to local environmental changes like proton transfer, perhaps they can be used instead for certain types of biological sensing, namely detecting local changes in membrane potential. Yakovlev then noted that other types of existing microscopy can potentially be used to control quantum behavior. Stimulated emission depletion (STED) microscopy places molecules in a particular excited state prior to fluorescent emission, making it potentially useful for probing local quantum dynamics. Dark state spectroscopy could also be used to give some chemical specificity due to suppression of absorption via quantum interferences, as quantum interference allows selective excitation of particular bonds. As a final note on potential applications of non-classical probes, Yakovlev noted that potassium ion channels exhibit indicators of quantum phenomena. The high degree of speed and selectivity and the mandate of only three ions for transport all point to underlying non-classical processes governing the channel's dynamics.

The final round of audience discussion brought up the need for a "killer app" for using entangled photons for imaging, and if there is any application that would bring about exponential improvement rather than just integer multipliers.

6.0 Panel 4: Possible Non-Classical Biological Systems

In the first presentation of the session, Yun Chen (Johns Hopkins University) demonstrated how quantum effects can be associated with mitochondria function as oscillation is an inherent property of the mitochondrial network under physiological conditions. She showed the possibility that these oscillations are related to electron transfer on the mitochondria membrane through collective electron tunneling for adenosine triphosphate (ATP) synthesis. Furthermore, she emphasized that there are molecular pumps preserving quantum coherence and acting as harmonic oscillators in mitochondria. As a method to prove quantum coherence in mitochondria, electronic paramagnetic resonance (EPR) can be utilized. Chen's opinion is meaningful in that quantum effect provides possible evidence regarding a biological mystery involving ATP synthesis. From the conventional point of view, when mitochondrial oscillations occur, electrons hop from molecule to molecule within the mitochondria to generate ATP. However, this could not account for the incredible speed of ATP synthesis. In a non-classical way, Prof. Chen demonstrated that the electrons exist in a quantum superposition and that they can move across the molecules at once from the quantum tunneling.

Valentina Benfenati (Institute of Organic Synthesis & Photoreactivity, National Research Council of Italy) discussed how brain function can be analyzed in a non-classical way. Benfenati showed that photostimulation of astrocytes using an infrared laser can enhance brain function through a quantum effect. The astrocytes are found to be involved in the onset of oscillatory synchrony, which is highly associated with brain function (i.e., cognitive processes). To relate brain function to the quantum effect, researchers have developed an experiment to measure the conscious coherent state using lasers. For example, two different lasers can be irradiated into two different regions of the brain. During the activation of various brain functions, such as consciousness, if quantum coherence is detected, a quantum effect modulates between cognitive or conscious processing. Benfenati also mentioned that cell communication by intracellular calcium signaling can be described in a non-classical way. It has been known that there is a quantum-like protectorate, composed of calcium ions trapped in the astrocytic syncytium, that can control the brain function (e.g., human consciousness). In this way, researchers can demonstrate how the brain processes a myriad of data in an efficient way that classical physics cannot explain.

Dan Sackett (National Institute of Child Health and Human Development, National Institutes of Health) posited how microtubules could be appropriate models for quantum behaviors. He mentioned that quantum coherence could occur on the microtubule surface, which is particularly dense with microtubules and polymer hair. Microtubules are composed of tubulin proteins that have non-polar regions containing electrons. These electrons can be entangled in terms of quantum mechanics, but it is not easy to prove. As Sackett noted, significant interest exists regarding the quantum effect in microtubules and its potential link to brain function. For example, quantum vibrations were recently discovered in the microtubules inside neurons. The microtubule vibrations can lead to the brain waves termed electroencephalogram (EEG) rhythms, which is relevant to both neurological conditions and cognitive processes.

7.0 Panel 5: Non-Classical Probes Applied to Biology

Theodore Goodson III and Gary Luker (University of Michigan) joined the Smart Sensing Workshop virtually to discuss their joint research using entangled photon spectroscopy for imaging at the cellular level. Because entangled photons can populate virtual levels that are not accessible through classical excitation, this method allows for "doing chemistry through spectroscopy," or determining how entangled photons interact with different molecules. Theodore Goodson and Luker discussed how this type of non-classical light could be used for cancer- and neuroscience-related applications. For example, the tumor environment is both complex and heterogeneous, composed of many types of cells in different biochemical states. Entangled photon spectroscopy can determine how each cell type responds to a certain cancer treatment by revealing changes in biochemical activity. Theodore Goodson and Luker also emphasized how it would be particularly advantageous to use this imaging modality for targeting cancer stem cells, which metabolize fatty acids differently than normal cells and even other cancer cells. Since each of the lipid components of the cancer stem cell metabolic cycle likely has a unique spectroscopic signature, it would be possible to identify cancer stem cells using those signatures and then redirect their metabolism to be less malignant. In the same vein, an additional exciting application would be to use entangled photon spectroscopy to alter astrocyte-neuron signaling. Not until recently has it been realized that glial cells provide more than just the "glue" for neurons; in fact, astrocytes have been shown to modulate synaptic signaling as is explained by the tripartite synapse theory.[1] Altering synapses by regulating astrocyte-neuron signaling could have significant implications for enhancing cognition and treating neurodegenerative diseases. In summary, Theodore Goodson and Luker proposed that non-classical spectroscopy can use low-intensity light to detect different cell types and, ideally, perturb cellular behavior.

The audience was excited by the prospect of entangled photon spectroscopy and asked several questions about the technicalities of this type of imaging. In particular, everyone agreed that it would be particularly advantageous to use quantum light in the three-dimensional environment, but for now, this is still part of the challenge that using non-classical light presents.

Peter Burke (University of California Irvine) gave the second talk and discussed using quantum technologies for pH sensing in the cytoplasm. To motivate this technology, Burke discussed how the metabolic activity of stem cells is unique because stem cells use more mitochondrial activity as they differentiate, whereas tumor cells follow the Warburg effect and use less mitochondrial activity. Moreover, since imaging mitochondria in detail is challenging due to their small size (generally < 1 pixel), changes in mitochondrial function can be detected by sensing intracellular pH because mitochondria pump ions across their membranes during oxidative phosphorylation. Measuring pH intracellularly also presents a challenge since currently no sufficient technology exists to accomplish this. Quantum sensors, on the other hand, would allow for real-time, *in vivo* imaging of mitochondrial activity. Burke and his lab are currently investigating the use of carbon nano-tubes or graphene to probe ion channel electrophysiology, which could provide a measure of mitochondrial function. Burke also emphasized how, in biology, function is always related to structure. He explained how mitochondria ultrastructure will change with corresponding shifts in mitochondrial metabolism. To investigate this, his lab is also using a GHz-capacitance-mode atomic force microscopy (AFM) to attempt real-time imaging of the ultrastructure. Since the structure-function relationship in organelles has hardly been explored, this area of research is novel and has significant potential impact, not only for cancer detection but also for mitochondrial disease, which affects one percent of all newborns.

As with the previous presentation, a technical discussion ensued to clarify how detection of mitochondrial metabolism works in practice. Burke explained that currently his lab looks for tiny changes in absorbance, since each small change is a proton being pumped through the electron transport chain.

Wolfgang Losert (University of Maryland) presented research on intracellular excitable systems and discussed the quantum state of biological systems. Losert asked the audience to think of internal signaling pathways and the cytoskeleton as excitable systems. While not a conventional view, Losert's lab has observed waves of assembly and disassembly – of both mechanical and biochemical systems – that occur naturally inside the cell. Moreover, the Losert lab has shown that the dynamics of these signaling systems can be quantified using an optical flow algorithm and controlled using surfaces with specific nano-topographies. Indeed, the dynamics of actin waves seem to be conserved across many cell types. To build on this work, the Losert lab has built a microscope capable of Broadband Coherent Anti-Stokes Raman Spectroscopy (BCARS) for the purpose of imaging actin dynamics. Actin shows a weak but detectable signal through BCARS, but culturing cells on ridged surfaces forces actin to concentrate along the ridge tops, so Losert is hopeful that his lab will be able to use this non-classical optical technology to detect stronger actin signals. Additionally, since cells likely do not encounter many flat surfaces *in vivo*, using surfaces with sub-micron sized ridges is more physiologically relevant. Finally, Losert ended by emphasizing how non-classical microscopy and novel quantum sensors would improve the ability to detect and, therefore, understand the intracellular dynamics of excitable systems.

When it came time for discussion, Losert received several questions about actin dynamics and excitable systems. He explained how the original purpose of using ridged surfaces had been to confine cells, but instead, his lab found that cells (and intracellular signals) could be guided along ridges. In a follow up question, he explained how the ridges may be the size of a collagen bundle, which many cells recognize as providing a path for locomotion.

The final talk of this session was given by Makhapa Makhafola (MINTEK). Makhafola, General Manager of Research & Development, explained how MINTEK is a national mineral research organization that creates biosensors (using gold nano-particles) as well as other nano-scale sensors. While MINTEK's biosensors are primarily used to test for HIV and Malaria, there are many other applications, including assessing food quality, water cleanliness, or trace pollutants. Given the other presentations, Dr. Makhafola stated how MINTEK's biosensors could prove useful for academic labs. Additionally, given MINTEK's success with HIV test kits using gold nano-particles, the organization is considering developing other kits, such as for the detection of breast cancer. MINTEK's technology could provide an excellent opportunity for collaboration with academic labs that have expertise in cancer detection and cancer cell metabolism. Since MINTEK is dedicated to the development of mineral technology and technology transfer, Makhafola encouraged the audience to consider MINTEK as a collaborator and/or partner since many opportunities exist for using nano-technology and nano-materials in academic research.

7.1 General Discussion

A lively discussion ensued, and Makhafola answered several questions about MINTEK's processing and post-processing capabilities. MINTEK currently makes gold nano-particles using state of the art facilities, always seeking to optimize the process, and they are also producing quantum dots. Finally, Makhafola clarified that MINTEK's goal is to provide support for any type of research, whether it is in the concept stage or the development stage.

7.2 References

[1]Eroglu, C. and B. A. Barres (2010). "Regulation of synaptic connectivity by glia." Nature **468**(7321): 223-231.

8.0 Panel 6: Smart Sensing: Mixing All the Ingredients

The first talk of this session was presented by Ms. Rosie Hicks (ANFF), CEO of the Australian National Fabrication Facility (ANFF). ANFF is a company whose mission is to provide world class nano- and micro-fabrication research facilities and services across Australia in the form of eight specialized nodes that link 19 Australian universities and the Commonwealth Scientific and Industrial Research Organization (CSIRO). Ms Hicks went on to describe the long, on-going collaborative efforts using ANFF facilities, which have brought Australian researchers and leading researchers from other countries (e.g., US and Italy), to find new discoveries and generate novel innovations. In addition, she went on to describe a key partnership with the Air Force Office of Scientific Research (AFOSR) in enabling these technical exchanges and the importance that exposure to researchers with different backgrounds and experiences can lead to transformative breakthroughs.

Next, Prof. Chenzhong Li (NSF) spoke from the perspective of his dual role as a Professor in the Department of Biomedical Engineering at Florida International University (FIU) and as Program Director at the National Science Foundation (NSF), where he leads the Nano and Biosensing Program. Prof. Li started his talk on his own research work in smart sensing in the context of bioelectronics, namely the role of electricity and electrical signals can play in biosensing and how one can leverage these elements from a 'non-classical' perspective and its potential for integration into smart sensors. He pointed out that our bodies are made up of living cells and yet electrical signals are constantly being generated and sensed through these live systems as well as influenced by external signals. He next described how nucleic acids could be utilized as a biosensor through impedance measurements but also how at the cellular level the manipulations of cells via electric fields and how that has implications as a possible non-conventional cancer treatment. Prof. Li also emphasized the importance of system integration such as microfabrication to fabricate a robust smart sensor.

Prof. Li next gave an overview of other biomedical activities at NSF such as biophotonics, regenerative medicine and advanced biomanufacturing. He pointed out coordination involving multidisciplinary field, such as smart sensing, is important not only between programs within NSF but with other agencies like AFOSR and NIH. These types of coordinated activities create more robust research communities by leveraging a larger and diverse talent pool.

Dr. M. Jones Papo (MINTEK) manages the Advanced Materials Division at MINTEK and gave an overview of the importance in designing hybrid material systems/structures for smart sensing needs. He also emphasized an innovative integrated approach across disciplines along with engineering principles to design functionality in responsive materials and pointed to systems in nature as model systems that can generate new innovative and novel biologically inspired materials. Dr. Papo highlighted a joint initiative between South Africa, US, Italy and Australia in this area that will have implications in aerospace, energy, and health-human performance fields. He gave an example of creating an eco-friendly, highly efficient, rechargeable battery using traditional transition metal oxide based materials but also incorporating redox-active biomolecules as a novel electrode material. He concluded his presentation with an observation that biological systems evolved of a long period of time in pursuit of optimization. Taking advantage of their functions can offer insights in the design of advance materials for smart sensing.

The final speaker of this session was giving by Prof. Shashank Priya (Penn State), Associate Vice President for Research, Director of Strategic Initiatives, and Professor of Materials Science and Engineering at Pennsylvania State University. Prof. Priya's research is focused on developing bio-inspired materials, understanding the complex nature of properties in these materials, and once this understanding has been achieved, utilizing them to invent unique applications. In his presentation, bio-hybrid organic semiconductor devices for smart sensing wer used as an example of his research focus. He also highlighted the US Air Force sponsored research of incorporating DNA into organic electronics (e.g., light emitting diodes and transistors) in order to better understand and control transport properties of DNA. While seemingly not intuitively obvious, the incorporation of DNA into these organic electronic devices led to remarkable improvements in both photovoltaic efficiencies and on/off ratios in the dark. Dr. Priya next talked about another area of bio-inspired sensing with the fabricating of an artificial statocyst sensors via 3D printing. In nature, a statocyst is a gravitational sensor (i.e., inertial guidance) in some aquatic invertebrates such as jellyfishes at allow it to sense acceleration and thus allows a jellyfish to help maintain balance and direction.

9.0 Summary

Over the two-day workshop, a small group of thought leaders explored the connection between non-classical behavior found in biological functions and whether such connections could be insightful as well as leveraged in the area of smart sensing. While the group came from very diverse backgrounds and provided unique perspectives, there was a tremendous amount of excitement and enthusiastic discussions on the prospects of converging these topics together and establishing a nascent field. Each of the six sessions during the workshop had lively questions discussions both during and after each session. The participants left the meeting feeling positive (almost confident) that both transformative discoveries but more importantly innovative applications will emerge if this area of research. The topics discussed under the 'non-classical' behaviors were deliberately broad ranging from quantum mechanical and biophysical processes to collective behaviors that incorporated a mixture of chemical, electric, magnetic, mechanical, and/or optical phenomena to propose novel sensing mechanism and applications.

Nonetheless, there were many outstanding questions/issues that were raised which will require further discussions to help catalyze this emerging field. Some of these include:

- *Creating tools (and environments) where specialists from both the 'soft' and 'hard' sciences can come together to both teach and further develop these better tools. For example, could improved pH, ion, and water transport sensors be fabricated for studying biological functions;*
- *Further examination of promising non-classical phenomena such as quantum entanglement as a probe (e.g., entangled photon spectroscopy or diamond NV- centers) of biological system;*
- *Exploring collective behavior phenomena as a orthogonal mechanism to enhance and provide improved resilience in smart senor;*
- *Integrating multiscale approaches may be necessary to bridge some of the biology/physics gaps;*
- *Broader funding opportunities that help span these multiscale phenomena and allow for examination that bridge quantum (molecular) effects up to larger physiological effect; and*
- *Establishing an infrastructure/environment to help better facilitate similar interests and communications amongst 'soft' and 'hard' researchers.*

This workshop initiated and stimulated the discussion on blending smart sensing with non-classical effects in biological systems and to spur a collaborative research network among physical scientists/engineering and life scientists.

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