Biophotonics: the big picture

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Abstract. The 5th International Conference on Biophotonics (ICOB) held April 30 to May 1, 2017, in Fremantle, Western Australia, brought together opinion leaders to discuss future directions for the field and opportunities to consider. The first session of the conference, “How to Set a Big Picture Biophotonics Agenda,” was focused on setting the stage for developing a vision and strategies for translation and impact on society of biophotonic technologies. The invited speakers, panelists, and attendees engaged in discussions that focused on opportunities and promising applications for biophotonic techniques, challenges when working at the confluence of the physical and biological sciences, driving factors for advances of biophotonic technologies, and educational opportunities. We share a summary of the presentations and discussions. Three main themes from the conference are presented in this position paper that capture the current status, opportunities, challenges, and future directions of biophotonics research and key areas of applications: (1) biophotonics at the nano- to microscale level; (2) biophotonics at meso- to macroscale level; and (3) biophotonics and the clinical translation conundrum.

Keywords: multiscale biophotonics; translational biophotonics; white paper; outlook.

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1 Introduction

Biophotonics—the science at the convergence of light and biological matter—has come a long way over the past three decades. Advances in light-based technologies have resulted in innovative and transformative tools to study and manipulate biological systems at the subcellular, cellular, tissue, and organ levels. Thus, biophotonics is expected to play a key role in next-generation diagnostic, analytical, and therapeutic modalities of the 21st century. This is clearly demonstrated not only by its rapidly expanding scientific literature and industrial growth, but also by several recent global initiatives that include biophotonics as a major cornerstone. Examples include the “National Photonics Initiative (NPI)” based in the United States, “Towards 2020—Photonics driving economic growth in Europe, Multiannual Strategic Roadmap 2014 to 2020” in Europe, and “PhotonicsSA Roadmap” in Australia. These initiatives have clearly identified the potential for biophotonics to address important societal challenges, including in human health through the development of medical devices for management of human diseases; in environment health by enabling highly sensitive devices for advanced pollution detection; and in food and agriculture through providing means for assessing quality control and safety. Recognizing the importance of light and optical technologies, the United Nations General Assembly declared 2015 the International Year of Light—a global initiative to raise global awareness of how light-based science and technologies promote sustainable development and provide solutions to global challenges in energy, education, agriculture, and health. Finally, advances in biophotonic technologies enable scientific discoveries by providing tools for life-sciences research. For example, Nobel prizes were awarded in 2008 “for the development of green fluorescent protein” and in 2014 “for the development of super-resolved fluorescence microscopy” both ground-breaking technologies that have revolutionized optical bioimaging and have enabled many advances in biological research.

Transdisciplinary discoveries in biophotonics have harnessed light-based imaging and sensing tools to capture information from biological processes over size scales ranging from events occurring at the single molecule, through to secondary and tertiary biological structures to subcellular and cellular anatomy, as well as in small animals and patients. We will divide the discussions that took place during the ICOB somewhat arbitrarily, into the nano-to-micro and meso-to-macro scales, recognizing that there is much in common but also differences between the methods and applications of cellular/subcellular and in vivo clinical imaging and sensing. The interdisciplinary drivers to capture biological data over these size scales arise both from the desire to stretch the fundamental limits of optical techniques and to address critical needs for tools to study the molecular origins of health and disease as well as medical diagnosis. There are inherent opportunities and pitfalls in the push and pull of these motivations. We will not discuss here the therapeutic applications of biophotonics, which has its own distinct features.

2 Biophotonics at Nano- to Microscale

Here, we consider key advances that are needed for biophotonics at the nano- to microscale.
2.1 Pushing the Limits

Normal and pathological processes usually present themselves on the macro- and systems-biology levels, but their origins are found at the single-molecule level, which is below the diffraction limit of light. These can take the form of genetic mutations leading to nucleotide base substitution that culminate in uncontrolled tumor growth, or lipid oxidation within the myelin membranes of the neuronal tracts of the spinal cord that cascades to create neuro-degenerative disorders like multiple sclerosis and motor neuron disease. Advances in super-resolution imaging, sample handling, and data processing are allowing some of these events to be visualized directly. However, just as these single molecular events are the sentinel origin of the disease, they occur within a complex biological milieu of contributing and counter-regulatory parallel and linked molecular events. Hence, capturing this discrete nanoto micromolecular information in the larger biological context presents major technical and computational challenges.

2.2 Searching for Rare Signals

Just as there is a quest for biological information at smaller and smaller size scales, there is a push to find the earliest, and hence rarest, origins of these processes. Again, the targets of interest occur in a complex environment, in which signal specificity and sensitivity are challenged to overcome background noise. These sensitivity limitations are being addressed in several ways: brighter probes, more sensitive detection, uniqueness of signal against biological background, separation of the signal from the noise, and data processing. Through the use of synthetic biology and discovery chemistry, brighter fluorescent and luminescent proteins, enzymes, and chemical probes are being created. Probe emissions can be captured by instruments with enhanced collection efficiencies, coupled to powerful excitation approaches such as multiphoton excitation. Other approaches employ unique optical signals that are not normally present in biological systems, such as the use of photonic crystals that provide a way to visualize and sense signals where noise has been heavily suppressed. Again, here adding the time dimension is beneficial, since much of the background photoemission from biological fluorophores occurs very rapidly (∼30 ns) so that the signal probes with lifetimes > ∼ 30 ns can be acquired with minimal background. Finally, extracting signals from noise using advanced data processing techniques is also proving beneficial in isolating these rare events.

3 Biophotonics at Meso- to Macroscales

Biophotonics across the meso- to macroscales encompasses a wide range of technological and application-specific opportunities and challenges. These scales include in vivo imaging of very small living organisms such as C. elegans, Drosophila, zebrafish, and African frog tadpoles (Xenopus laevis), as well as larger animals such as rodents and rabbits. Also included are the use of human subjects and clinical trials.

3.1 Current Technological Advances

We identify eight primary areas of technological advances:

1. Imaging speed is rapidly advancing, such as swept-source OCT systems with MHz axial scan rates, requiring also the corresponding use of faster detectors. Computational imaging has also expanded, leveraging advances in high-performance computing, parallel computing, and processing and display hardware.

2. Detectors and detection systems are becoming more sensitive, enabling better signal collection with lower noise.

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4. Optical imaging techniques are emerging that employ multimodal physical processes as sources of image contrast and so enrich the information content, for example, photoacoustics, nonlinear imaging, targeted and activatable molecular probes, and biomechanical properties (such as elastography).

5. There is increasing use of multimodal optical imaging systems involving the simultaneous collection and coregistration of images from multiple contrast mechanisms over space and time. This also drives research into how these contrast mechanisms relate to one another and how using “image math” to generate weighted combinations of optical contrast to reveal information about the tissues of interest: ultimately, the sum is greater than the parts.

6. While, for obvious reasons, noninvasive imaging methods are usually preferred, advances in beam delivery systems have made optical imaging of one form or another feasible throughout all regions of the body, albeit sometimes requiring somewhat invasive procedures. In many scenarios, however, such as during surgery with exposed tissues, biophotonics imaging can certainly shine.

7. Biophotonic imaging will reap enormous benefit from recent advances in machine- and deep-learning algorithms. Coupled with the computational power that is now available and the immense information content of our images, we will continue to see how deep-learning will reveal patterns in images and will couple with computer-aided-diagnosis to reveal patterns that are otherwise not visualized.

8. Finally, the convergence of biophotonics with nanosciences and nanotechnologies is rapidly expanding the ability for highly multiplexed detection/imaging as, for example, in surface-enhanced Raman scattering (SERS) nanoparticles as well as periodic array-based substrates for SERS and surface-enhanced spectroscopies in general. Combined optical and radiological imaging (e.g., positron emission tomography and MRI) is now also becoming possible through the use of multimodal nanoparticles.

3.2 In Vivo Preclinical Research

Biophotonics has important roles to play in addressing the 3Rs (replacement, reduction, and refinement) as the framework for humane research in animal models of health and disease. For example, currently a typical drug response study involves at least four dose levels (vehicle, low, medium, and high),
the capture of pharmacokinetic and pharmacodynamics data at 6 time points, be conducted in both sexes, include separate sample collection for protein, imaging, and mRNA endpoints, and account for experimental biological variance that may require up to 12 animals per group. This equates to a $4 \times 4 \times 2 \times 3 \times 12 = 1728$ animals. This is not only ethically challenging, but also is time and labor intense and expensive. However, in the near future, the types of tools highlighted above will be translated into so-called “behaving” in vivo models, enabling multimodal and multiplex measurements in anatomical compartments and within the primary organs that previous in vitro and ex vivo systems could only crudely model. This could allow up to a 1 to 2 orders of magnitude reduction in total experimental animal use and marked expansion of within-subject statistical power.

3.3 Challenges

With these major advances come challenges, and we identify five that would benefit from further research and new thinking.

1. There is a severe inherent trade-off between optical imaging depth and spatial resolution. While we enjoy beautiful high-resolution images of cells and subcellular structures, achieving this at meso-macro scales is confounded by light scattering. Are there technologies and algorithms that can potentially reconstruct molecular and cellular details or provide signatures even deep within living organisms?

2. Robust solutions do not currently exist for connecting images and data across large spatial scales, from sub-mm to tens of cm. This challenge may be addressed by multimodal zoomable imaging systems or instruments providing rapid scanning of large areas/volumes of tissue, followed by computational processing and reconstruction of images with micron-scale features.

3. While beam delivery devices continue to advance, there is no robust fiber-optic endoscope system capable of high-resolution nonlinear imaging, due primarily to the optical dispersion and continual motion and bending of optical fibers that degrade ultrafast (fs) laser pulses that are used routinely in nonlinear microscopy and nanoscopy.

4. As with micro/nanoscopy, data volume, analysis, and management represent a continuing challenge, even with current advances in computing power and data storage. More intelligent systems are needed to determine which images/data contain the highest information content and which can be discarded.

5. In a related way, such intelligent systems are needed to transform image data into useful knowledge for decision making, whether that be to discover a fundamentally biological process or to detect and diagnose disease in a patient.

3.4 Promising Targets in Biology and Medical Research and Applications

At the meso-macro scale, there are several promising trends, including the following. Intravital imaging will play an increasingly important role in discovery of biological processes, both in homeostasis and in disease. Either label-free or coupled with exogenous molecular probes, we will see more intravital imaging applications to elucidate the dynamics of biological processes that cannot be replicated in cell culture systems. Additionally, we will see an increasing use of intravital optical imaging in humans to assess health and search for evidence of disease. There will be an increasing role for biophotonics imaging of patient-derived xenografts and organoids, as well as in organs-on-a-chip. As the biotechnology of these living structures advances, there will be a strong need to noninvasively visualize their microstructural, molecular, metabolic, and functional changes and relate this information to observations and processes in vivo. Beam-delivery systems such as internal optical “pills” and tethered optical probes will enable visualization of the inner workings of biological processes and disease detection. Optogenetics is expected to expand beyond its origin in neuroscience and become a general technique by which molecular and cellular processes can be stimulated, manipulated, and controlled with light. Because biophotonic technologies for imaging or diagnosis can be exceptionally compact and portable compared with other nonoptical techniques, we will see increasing miniaturization of platforms for point-of-care and point-of-procedure applications. These will help to push the diagnostic capabilities of biophotonics closer to the front-line of our healthcare delivery and systems, including in the developing world. Biophotonics will also play increasing roles in drug discovery and efficacy, particularly for personalized medicine. Thus, while most pharmacotherapies are designed for the average patient population, it is recognized that each individual metabolizes and responds to drugs differently. Biophotonics systems will be developed to track drug delivery and distribution, as well as the resulting molecular and cellular responses, enabling more patient-specific dosing and treatment. With the challenges associated with regulatory approvals for contrast agents or nanotechnologies in medicine, there will be increasing interest in label-free imaging methods using both linear and nonlinear interactions that allow more rapid translation into first-in-human studies and subsequent clinical trials.

4 Scale-Independent Issues

There are several key issues in biophotonics that apply across all scales and fields of application.

4.1 Multimodal Data Collection

As fundamental events in complex biological systems are explored, there is mounting pressure for parallel streams of mechanistic information and there have been corresponding fundamental advances in multimodal data collection, with systems comprising several imaging and sensing modalities. These approaches are beginning to enable quantitative studies of complex events to test a priori mechanistic hypotheses. Hypothesis-free, sample-phenotyping approaches are also available through the use of techniques such as Raman and tissue autofluorescence acquisition. In addition, multiplexing of target signals within a specific measurement technique is also often required and this has driven the creation of a wide range of synthetic biology and chemically derived, spectrally distinct fluorescent and luminescent probes for parallel sensing and imaging. However, the ability to separate these probes spectrally is limited, so that techniques that also incorporate time-encoding hint at a future...
where tens of targets could be measured simultaneously using single excitation and detector pairs.14

4.2 Big Data, Machine Learning, and Response Phenotyping

As in many other fields, there has been an explosion in the size of the datasets generated in advanced biophotonic techniques. Current analytical approaches only scratch the surface of the potential information that is contained in such datasets. Machine-learning techniques will allow extraction of more and more subtle and complex signals and identification of events that are not a priori anticipated. Inherent in current machine-learning is the difficulty to define where the specific signal is coming from in, for example, complex neural networks. This will necessitate methodologies that can validate sample phenotyping and classifications rather than just specific analyte quantification, since data from complex n-dimensional spaces will be used instead of restricted spectral ranges as currently employed. While these approaches bring immense potential analytical power, they risk a lack of defined specificity and assay transference, and any associated sample signal drift needs to be remedied in line with validation approaches. While computational imaging with machine learning and deep learning has huge potential, there is an urgent need for more “ground-truth” identifiers against which to train the enormous volumes of image data that can be collected. Our current ability to generate these ground-truths is orders-of-magnitude slower than the rate at which we can generate images from our systems.

4.3 Light-Triggered Events

The development of optogenetics has provided a range of light-based methods to control the ionic conditions of selected cells. This was originally motivated by the neurosciences but now extends to include genetically encoded light-based control of, for example, GPCR function, enzymatic activity, gene expression, and protein–protein interactions across multiple tissues. These tools will continue to advance alongside emerging theranostic technologies that are allowing spatial and temporal targeting of exogenous material. Coupling them with multimodal and/or multiplexed data collection will allow interrogation of biological processes with microscopic resolution, while the use of fiberoptics enables investigations at the microscale in remote anatomical locations within complex and behaving biological systems.

4.4 Compatibility Does Not Equal Toxicity

As the tools and approaches to interrogate biological processes expands, the issue of the “observer effect” mounts in relation to both the use of exogenous probes and light into the biological system, whether this be a single cell or a living patient. Three laws of pharmacology can be applied here: (1) toxicity—everything is toxic, it all depends on the dose; (2) pharmacodynamics—what does the biological system do to the agent? and (3) pharmacodynamics—what does the agent do to the biological system? The common assumption to date has been that, if the nanomaterial, light source or chemical probe do not cause cell death based on a crude cell-viability (e.g., MTT) assay then there are no significant observer effects. However, this ignores rules 2 and 3 above. In exploring the use of existing and photoactive materials, it should be recognized that they are foreign to the body (xenobiotic) so that there may be some confounding observer effects. The introduction of light into “dark” places in biology is a potential stimulus that can be toxic or alter the biokinetics and biodynamics.

4.5 Integrity of Scientific Reports and Experimental Replication

One of the greatest threats to scientific progress in the biosciences is the lack of experimental replication. Putting aside intentional falsification, the more pressing issue is the failure to replicate otherwise sound experimental practices between laboratories and across in vitro to in vivo boundaries. These issues should be of major importance to the biophotonics community. The appropriate development and deployment of biophotonics tools, intimately coupled with thorough education and training in the science underpinning the technology can reduce this crisis of irreproducibility. At the very least, our technologies must not add to the problem. This is no small task, as discipline boundaries of knowledge, understanding and language need to be bridged and eventually dismantled. To adequately master this challenge, large teams of scientists working across and between disciplines need to be funded to collaborate for extended periods of time to tackle these “grand challenges.” Such efforts should reinforce surrounding capabilities and create a legacy of trainees and collaborations that can sustain the field into the future. Finally, this knowledge and technology needs to be disseminated globally across geographic boundaries, necessitating ongoing support of free scientific knowledge sharing, and transnational interactions and collaborations.

5 Biophotonics and the Clinical Translation Conundrum

5.1 Translational Versus Transformational Research

The clinical translation of biophotonics technologies faces many hurdles, validation points, and adoption issues. While translational research is the term generally used to describe bench-to-bedside progression of technologies, we contend that far more effort is needed than to go from the patient-to-the-population, or to truly conduct transformational research. This encompasses benchmarking the new technology against the current standard-of-care processes and technologies, as well as fostering their adoption and integration into the clinical workflow and the healthcare system. Whether it be translational or transformational research, finding and defining clinical problems that may be amenable to biophotonics solutions are critical and require astute observation and interdisciplinary listening skills. Such skill development and training needs to be part of every graduate or professional training program. With open ears and an open mind, the clinical problems in need of technological solutions, such as those provided by biophotonics, will be unending. To assist in this process, basic and applied scientists and engineers need to be physically immersed in the cultures and environments where these problems occur, namely in hospitals and clinics and across our healthcare systems. There they will begin to find solutions from their knowledge banks and training that come from seeing and experiencing the clinical problems first hand and in an ongoing way, providing new perspectives that are not bounded with established disciplines.
5.2 Performance Versus Practicality

The translation of technology is often challenged by the opposing goals and needs between the scientists and engineers developing the technology and the clinicians and healthcare providers who will eventually use it. The former love the complexity that often fuels their creativity and innovation. They will also often deliberately increase the complexity of the technology or the system under study in order to maximize performance. Unnecessary complexity and excessive performance, however, may disincentivize the potential clinical adopters, because of factors such as the added training requirements to operate and maintain these systems and to read, analyze, and interpret the complex and/or voluminous data generated, as well as the cost. For medical devices and technologies, simplicity is often the key to adoption: the technology needs to be just complex enough to solve the clinical problem. The pulse oximeter is perhaps a perfect example of using a fundamental biophotonics principle to non-invasively measure the oxygen saturation in the blood (along with the heart rate). This simple measurement is so clinically valuable that it is regarded as a “vital sign” in patient management, along with heart rate, respiration rate, body temperature, blood pressure, weight/height, and level of pain.

5.3 Driving Forces

The driving factors for new medical technology, including biophotonic devices and imaging technologies, are largely consistent across all of medicine and surgery: improved patient outcomes, healthcare cost reduction, increased efficiency, reduction of workload, and efficient management of large amounts of data from different types and from multiple sources. Medical devices and algorithms require regulatory approval, and having precenders or predicate devices on which to build for regulatory approval is advantageous if not essential. We all acknowledge and thank our colleagues who have first driven new technologies through this regulatory process, often against many hurdles and over years of effort that have streamlined the path for subsequent devices and approvals. We must seek out programs and experienced colleagues to inform and teach others about this process and develop clear procedures and pathways to more rapidly move technologies from laboratory prototypes through clinical and commercial prototypes to products. Finally, seeking and establishing medical billing codes for the procedures and protocols that will use the technologies is essential, since few physicians, hospitals, or healthcare systems will adopt new technologies if reimbursement is not in place.

5.4 Education and Training

The technology developed today, with effort and perhaps some luck, will be adopted, integrated, and used in standard-of-care practice for years, perhaps decades, from today. In parallel, there needs to be a concerted effort within our medical schools and allied-health colleges to educate and train the next generation of physicians who will be knowledgeable, skilled and comfortable using these new technologies. In fact, beyond simply raising familiarity and skills for new technology, our future healthcare professionals need to be educated to think more fundamentally about technology, how it interfaces with medical practice and patient care, and how it can be used to improve individual care and the healthcare systems. To this end, many academic institutions are developing innovative medical education programs that now include opportunities in bioengineering, information sciences, genomics, and personalized medicine. For example, the University of Illinois at Urbana-Champaign in conjunction with Carle Foundation Hospital, has established the Carle-Illinois College of Medicine, perhaps the first engineering-based medical school that will infuse engineering principles into every course in the medical curriculum and require technological and information science experiences and projects. This new curriculum will also train medical students to identify technological solutions to the medical problems that they and their patients face, to innovate and drive entrepreneurial projects forward from their patient-care experiences and training, and ultimately to improve on the compassionate care that they can deliver. A further example is the Master of Medical Photonics curriculum at the Friedrich Schiller University in Jena, Germany. The goal of this interdisciplinary program, which brings together physical scientists and life scientists and medical professionals, is to prepare the student for a science-based and research-oriented professional activity in the field of medical optics and photonics. An additional goal is to provide the basis for further training programs within or outside the university, in the course of which students will acquire in-depth knowledge of theory, methodology, and systems from the fields of biology, medicine, mathematics, chemistry, and physics. These are prerequisites for experimental capabilities necessary in medical biophotonics. Accordingly, the students receive special training in selected areas such as microscopy, spectroscopy, and diagnostics as well as in current clinical applications of photonic techniques to enable the graduates to apply and further develop optical methods in biomedical research and clinical application. This program also allows extension to the doctoral level, as well as gaining experience in technology-oriented companies in the optics, medical devices, and life science sectors, for which high demand is expected in the coming years. For medically qualified physicians, the master’s degree course can be the prerequisite for the double MD/PhD qualification.

6 Conclusions

As described above and underscored during the discussions at the ICOB conference, the applications of biophotonics technologies have evolved from their unique ability to interrogate biological systems at multiple scales from nano-micro to macro. Their applications are already very diverse. In particular, their utilization in medical diagnostics and therapeutics has expanded tremendously. By diligently addressing the barriers to clinical translation, it is anticipated that biophotonics-enabled medical devices and agents will become ubiquitous tools in clinical management of human diseases. Affordable light-based technologies and advances in photonic technologies and applications of photonic techniques to enable the graduates to apply and further develop optical methods in biomedical research and clinical application. This program also allows extension to the doctoral level, as well as gaining experience in technology-oriented companies in the optics, medical devices, and life science sectors, for which high demand is expected in the coming years. For medically qualified physicians, the master’s degree course can be the prerequisite for the double MD/PhD qualification.

An additional enabling of biophotonics and applications is the adoption of photonic devices used in other domains. For example, Google glasses that were initially developed for military applications are now adapted for surgical video streaming and navigation. This trend is likely to accelerate as the demand for higher and higher performance consumer products drives research and development of novel photonic techniques and technologies.
Although the historical impact of biophotonics was primarily in healthcare, with one of the earliest Nobel Prizes being awarded to Niels Finsen in 1903 in recognition of his contribution to the treatment of diseases... with concentrated light radiation, whereby he had opened a new avenue for medical science (for which he used a huge carbon-arc lamp from a lighthouse: technology translation indeed!), the current applications encompass a much wider base. Promising areas are environment control and monitoring, and agriculture and food quality. These applications are made possible by adapting existing photonic technologies to address needs in biotechnology, analytics, and sensing (biosensors), as well as integration into drones for remote environmental monitoring. The increase awareness among investors and governmental agencies about the important role that biophotonics can play in the development of novel cost-effective products is expected to also drive new advances: the 2017 market for biophotonics is forecast to be US$36.8 billion and to reach US$59.9 billion by 2022.

A new and exciting opportunity for the application of biophotonics is in human space exploration. In particular, the International Space Station is a unique laboratory for studying human responses to first-order changes in the environment, such as the removal of gravity. [23] Crews now spend 6 to 12 months in low-Earth orbit under microgravity conditions, which results in significant alterations to structural and functional physiology, not only in humans but also in small animals and plants. Studying these responses can illuminate underlying regulatory and adaptive principles, ranging from molecular and cellular to biochemical and transport-system biophysics. Biophotonic techniques offer unique capabilities in this domain due to their rich information content and the compactness and high robustness of the technologies.

The dynamics of the biophotonics workforce is also notable. The field demands scientists with a multidisciplinary education across a wide spectrum of disciplines, requiring not only a solid background in physics, engineering, and optics but also a working knowledge of laser–tissue interactions and general knowledge of biology, anatomy, and physiology. While there has been a shortage in the past of scientific and technical personnel with such broad interdisciplinary experience, sustainable efforts in education and training over the past two decades have resulted in a new generation of professionals able to accelerate biophotonics research and to fill the growing number of industry positions.

We are fortunate to live at a time of enormous potential of biophotonics to impact and improve on the quality of life for so many; not only the patients in our hospitals and clinics, but also more globally. Biophotonics will certainly play an important role in the revolutionary changes we expect to see in healthcare and in addressing other global challenges related to safety of the environment and food, agriculture, and possibly in the near-distant future in space exploration. Moreover, advances of biophotonics tools able to monitor cellular processes with molecular resolution and sensitivity have the potential to unravel complex mechanisms and processes and lead to untold scientific discoveries in biology and medicine.

Disclosures

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