Welcome to the "São Paulo School of Advanced Science on Modern Topics in Biophotonics"!

This school was planned to provide a rich environment for scientific discussions on modern optical technologies applied to Life Sciences. We would particularly like to encourage the active participation of students and young researchers on all school activities.

We have selected a wonderful group of speakers, leaders on their scientific fields, to present basic to applied aspects of current biophotonics techniques.

Finally, we would like to thank our sponsors, invited speakers, and all participants for making this event a promising one.

Enjoy the event and these days among this exciting group of people.

Sejam bem-vindos!

Organizing committee
Cristina Kurachi
São Carlos Institute of Physics - University of São Paulo

Rickson Mesquita
Institute of Physics - University of Campinas

Vanderlei Bagnato
São Carlos Institute of Physics - University of São Paulo

Lilian Moriyama
São Carlos Institute of Physics - University of São Paulo

Natália Inada
São Carlos Institute of Physics - University of São Paulo

Sebastião Pratavieira
São Carlos Institute of Physics - University of São Paulo

Program

1st Week

	20/03/2019	21/03/2019	22/03/2019	23/03/2019	24/03/2019
8:30-9:30	Registration		Course 1	Visit to UNICAMP labs	
9:30-10:00	Welcome	Course 1	Course 3		
10:00-11:00	Course 1 – V. Tuchin	Course 3 – CR Mendonça	Poster presentation 1		
11:00-12:00	Lecture 1 – D. Zezzel	Course 3			
12:00-13:30	Lunch	Lunch	Lunch		
13:30-14:30	Course 2 – M. Pereira	Course 2	Course 4		BBQ
14:30-15:30	Lecture 2 – K.T. Oliveira	Lecture 4: A. Caires	Course 4		
15:30-16:00	Coffee-break	Coffee-break	Coffee break		
16:00-16:40	Lecture 3 – S. Pratavieira/LT Moriyama	Students presentation 1	Lecture 4: Rickson		
16:40-17:30	SPIE Student Chapter	Course 4 – A. Yodh	FAPESP Lecture: H. Chaimovich		

2nd Week

	25/03/2019	26/03/2019	27/03/2019	28/03/2019	29/03/2019
8:30-9:30	Course 5 – M. Wainwright	Course 5	Course 5	Course 11 – A. Zanardi	Course 11 – A. Zanardi
9:30-10:30	Course 6 – G. Zheng	Course 6	Course 6	Lecture 9 – Overview BiophotonIFSC research	Visit to Optics Group labs
10:30-11:10	Lecture 5: L Bachmann	Course 8	Course 10	Course 10	
11:10-12:10	Course 7 – A. Scherz	Course 10– D. Boas	Course 7	Lecture 10: D. Milori	
12:00-13:30	Lunch	Lunch	Lunch	Lunch	Lunch
13:30-14:30	Course 8 – L. Lechuga	Course 8	Course 7	Discussions for potential collaboration candidates	Overall discussion
14:30-15:30	Course 9 M. Baptista	Course 9	Lecture 8: A.G. Salvio		Closing remarks
15:30-16:00	Coffee-break	Course 9	Coffee-break		
16:00-17:30	Students presentation 2	Coffee-break/Poster session 2	Students presentation 3	Coffee-break/Poster session 3	

Invited lecturers

COURSE 1

Valery V. Tuchin is a Professor and holds the Chair/Institute of Optics and Biophotonics of Saratov State University (National Research University of Russia). He is also a Head of Laboratory on Laser Diagnostics of Technical and Living Systems of Institute of Precision Mechanics and Control of the RAS, a supervisor of Interdisciplinary Laboratory on Biophotonics of Tomsk State University (National Research University of Russia) and Laboratory of Femtomedicine at University of ITMO, Guest Professor of HUST (Wuhan) and Tianjin Universities of China, and Adjunct Professor of the Limerick University (Ireland) and National University of Ireland (Galway). His research interests include biophotonics, tissue optics, laser medicine, tissue optical clearing, and nanobiophotonics. He has published more than 700 papers (Web of Science), 27 monographs and textbooks, and 60 book chapters. Prof. Tuchin is also a holder of more than 50 patents. He is a Fellow of SPIE and OSA, has been awarded Honored Science Worker of the Russia, Honored Professor of Saratov University, SPIE Educator Award, FiDiPro (Finland), Chime Bell Prize of Hubei Province (China), and Joseph W. Goodman Book Writing Award (OSA/SPIE). He has 20384 citations and h-index 66 (Google Scholar, August 6, 2018).

Tissue optics and optical clearing of tissues and cells

Many optical biomedical spectroscopic and imaging technologies suffer from a low penetration depth of a probing beam and blurring of images caused by light scattering and absorption. One of the robust ways to overcome these problems is to use a so-called tissue optical clearing (TOC) method. TOC can be provided by impregnation of tissue or cell by biocompatible optical clearing agents (OCAs) – this is an immersion optical clearing (IOC), by a local mechanical compression (squeezing or stretching) – this is a mechanical optical clearing (MOC), or by using optical adaptive system and intensive computations. The main goal of this set of lectures is to overview fundamentals and advances of tissue optics in the context of TOC, and to demonstrate a wide range of biomedical applications of TOC beneficial due to increase of light beam probing depth and image contrast of human and animal tissues. The enhancement of optical spectroscopy and imaging of living tissues and cells using different optical modalities working in a broad range of wavelengths from UV to terahertz will be demonstrated. Water transport and modification of tissue mechanical properties under OCA action such as reversible dehydration and shrinkage, balance of free and bound water will be analyzed. The IOC method explores three major concepts: refractive index matching of scatterers and surrounding medium, reversible dehydration caused by OCA osmolarity, and reduction of hydrodynamic radius of scattering particles at their interaction with OCA molecules [1-10]. As OCAs a variety of hyperosmotic, cryogenic, x-ray contrast and metabolic liquids, such as glycerol, PEG, glucose, fructose, sucrose, mannitol, dextrans, propylene glycol, ethylene glycol, iohexol (Omnipaque^{TM}), albumin, hemoglobin, and some others, will be considered and results of their testing will be presented.

The increase of light probing depth and image contrast will be demonstrated using spectrophotometry, OCT, photoacoustic microscopy (PA), *in vivo* PA flow cytometry (PAFC), upconversion nanoparticles luminescence, fluorescence, multi-photon autofluorescence, SHG and Raman microscopies, polarization and speckle imaging for a variety of human and animal tissues such as skin, fat, eye sclera, muscle, cerebral membrane, digestive tract tissue, cartilage, bone, blood vessels, and blood. The technologies for effective OCA delivery due to hidden free diffusion, local heating, enforced tissue

permeability (physical and chemical), OCA encapsulation, and blood and lymph vessel networking will be described. Impact of OCAs on tissue structure, free/bound water balance and blood microcirculation will be discussed. Experimental data on diffusivity and permeability of glucose, glycerol, PEG, iohexol (Omnipaque^{TM}) and other OCAs for normal and pathological tissues will be provided.
Combined tissue TOC technologies by using combinations of IOC, MOC, optical adaptive system and analytical (computational) ones will be discussed as well.

COURSE 2

Mariette M. Pereira obtained her PhD in Organic Chemistry in 1992 at the University of Coimbra and worked as Fellow Assistant Researcher at the University of Liverpool in 1993 and University Autónoma de Barcelona from 1997–1998. She has been Associate Professor with Habilitation at the University of Coimbra since 2007. She was Director of Chemistry Research Laboratory of Luzitin Lda, a pharmaceutical spin-off 2010-2015. Her current research interests are the synthesis of chiral binaphthyl based ligands for the development of asymmetric catalysts for carbonylation tandem reactions and development of sensitizers based on tetrapyrrolic macrocycles for biomedicinal applications and environmental catalysis. She has published ca. 150 peer-reviewed papers in international journals, 3 books, and 8 book chapters and is the inventor of several patents on the field of tetrapyrrolic macrocycles.

Tetrapyrrolic Macrocycle Based Photosensitizers: Synthesis and Clinical Use

The great advances achieved in photodynamic therapy of cancer (PDT) or photo-inactivation of microorganisms (PDI) are decisively due to the development of alternative synthetic methods for tetrapyrrolic macrocycles. These important class of natural biomolecules, like heme, chlorophyll and bacteriochlorophylls, can be easily modulated by chemical synthesis. The synthetic approaches for selected examples of tetrapyrrolic macrocycles, under advanced clinical phase/clinical use, will be presented. Their structural modulation to achieve the ideal properties to be applied in PDT or PDI will be discussed.

Development of Redaporfin® Photosensitizer: From Academia to Clinical Trials

The sustainable synthetic approach of a large family of stable halogenated sulfonated *meso*-aryl bacteriochlorins developed in the University of Coimbra- Portugal, and the pre-clinical studies performed in Luzitin, which led to the selection of the lead compound, will be presented and discussed. Moreover, the isolation of the amphiphilic 2,6-difluorophenyl *meso*-substituted bacteriochlorin atropoisomers and their structure/activity in PDT effect will be discussed.

COURSE 3

Cleber Renato Mendonça is the head of Photonics Group in the Institute of Physics of São Carlos, University of São Paulo and a member of several Councils and Commissions within the university. His research interests include nonlinear optics, ultrashort laser pulses, femtosecond laser microfabrication and coherent control of light matter interaction. He is also a referee in more than 20 scientific journals and was a visiting professor at the University of Bremen, Germany, and the University of Nantes, France. He has published more than 200 papers (Web of Science), which were cited more than 3000 times, has h-index of 35, and 5 book chapters. Professor Cleber is also a holder of 4 patents.

Ultrashort laser pulses and applications

These lectures will provide an introduction to some fundamental aspects of nonlinear optics and ultrashort laser pulses, with emphasis to their technological applications. The main goal is to provide a good understanding of the fundamentals and a comprehensive knowledge of the recent advances not only on the ultrashort pulses and nonlinear optical processes in materials, but also on the application for laser processing. Many practical examples are included throughout the talks, such as applications in optical storage, waveguides and biology. Specifically, this course will cover (1) basic concepts on nonlinear optics, (2) basic principles on ultrashort pulses, (3) a description of methods to investigate optical nonlinearities, (4) basic principles on laser microfabrication, and (5) various applications of the above techniques and methods.

COURSE 4

Arjun G. Yodh is the James M. Skinner Professor of Science and the Director of The Laboratory for Research on the Structure of Matter (LRSM) and its NSF-supported Materials Science and Engineering Center (MRSEC) at the University of Pennsylvania. His home department is Physics & Astronomy, he has a secondary appointment in the Department of Radiation Oncology in the Medical School, and he is a member of the Bioengineering Graduate Group. Dr. Yodh graduated from Cornell University (1981) with a BSc in Applied & Engineering Physics; he obtained his Ph.D. from Harvard (1986) under the guidance of Tom Mossberg, and he then spent two years at AT&T Bell Laboratories as a post-doc working with Steven Chu and Harry Tom. His current interests span fundamental and applied questions in condensed matter physics, medical and biophysics, and the optical sciences. Areas of ongoing research include: soft materials such as colloids, liquid crystals and other complex fluids, networks and films, carbon nanotubes, optical microscopy and micromanipulation, biomedical optics especially for functional imaging and monitoring of living tissues (brain, breast, muscle, spine, placenta) with diffuse light, photodynamic therapy and therapy monitoring with biophotonics, linear/nonlinear optics, and laser spectroscopy. He has published more than 430 papers (web of science) and holds 8 published patents and 5 pending patents. His papers have more than 35 000 citations (Google Scholar) and he has an h-index of 102 (Google Scholar).

Diffuse Optics

I will first describe the fundamental ideas that underpin a subfield of Biophotonics called “Diffuse Optics.” Diffuse Optics utilizes multiply scattered light to investigate deep tissues. My introduction will emphasize schemes to measure tissue absorption and scattering, fluorescence contrast, and blood flow; we will see that these parameters also permit study of tissue oxygen metabolism and autoregulation. Both monitoring and imaging concepts will be discussed in these contexts. Then I will describe pre-clinical studies and emerging clinical applications. Recent brain studies, for example, demonstrate potential for usage as a bedside treatment management tool in the neuro-ICU, especially for patients with traumatic brain injury and acute stroke; breast studies demonstrate potential of optical cancer therapy monitoring, especially for patients undergoing neoadjuvant chemotherapy prior to surgery.

COURSE 5

Mark Wainwright joined Liverpool John Moores University (LJMU), United Kingdom in 2005 and with the University formed the spin-out drug-discovery company Pharmalucia in 2008. He has been Professor of Chemotherapy at LJMU since 2011 and also has been involved in photosensitiser research and development since 1987. For the past 25 years he has worked mainly in photodynamic infection control applications (photoantimicrobials), especially concerning conventional drug resistance. He is currently one of the Editors-in-Chief of Dyes & Pigments, and in 2009 published the book "Photosensitisers in Biomedicine".

The Photodynamic Inactivation of Microorganisms.

This is a three-lecture course, based on the following general areas:
i) Concepts: photosensitisation; microbial physiology; toxicity.
ii) Strategies: selectivity; light application; conventional drug resistance.
iii) Applications: topical/local administration; other areas of infection control.

COURSE 6

Gang Zheng received his PhD in 1999 from SUNY Buffalo in Medicinal Chemistry. Following two years of postdoctoral training in photodynamic therapy at the Roswell Park Cancer Institute, he joined the University of Pennsylvania in 2001 as an Assistant Professor of Radiology, where he established the molecular imaging chemistry program and introduced photodynamic molecular beacons and lipoprotein-like nanoparticles. Since moving to Canada in 2006, his research has been focused on developing clinically translatable technology platforms to combat cancer. His lab discovered porphysome nanotechnology, which was named one of the "top 10 cancer breakthroughs of 2011" by the Canadian Cancer Society. His lab also discovered that on exposure to low-frequency ultrasound, porphyrin microbubbles form nanoparticles that possess the same optical and therapeutic properties as the original microbubble, and can be used simultaneously for imaging and drug delivery. Dr. Zheng is an Associate Editor for Bioconjugate Chemistry and a Fellow of the American Institute for Medical and Biological Engineering. Dr. Zheng has 10615 citations and h-index of 57 (Google scholar).

Addressing the translation hurdles in biophotonic nanomedicine

The overwhelming growth witnessed in the last two decades in the field of nanomedicine was largely triggered by the unique nanoscale material, physical and biological properties, represented respectively by (1) the ability to consolidate multiple functionalities into a single 'all-in-one' nanomaterial, (2) the inorganic nanophotonics, and (3) the idea that nanoparticles can preferentially accumulate in solid tumours due to the enhanced permeability and retention (EPR) effect. However, the unprecedented number of papers published in the field of nanomedicine remains disproportionately high relative to the number of nanoagents used clinically. It appears that these heralded nanoadvantages have increasingly become the Achilles' heel of clinical translation because multifunction-induced complexity contributes to new regulatory and scale-up challenges and inorganic nanoparticles' toxicity concern whereas EPR's clinical relevance is still controversial. This lecture series will share our experiences in addressing these translation hurdles in biophotonic nanomedicine.

My lab discovered porphysome nanotechnology, in which nanostructures are self-assembled from a single porphyrin-lipid building block. This well-characterized molecule can form nano- and micro-structures with different sizes, shapes, compositions and biophotonic properties. The resulting structures achieve many functions (photothermal, photoacoustic, fluorescence, photodynamic, PET, MRI, ultrasound, etc.) without the need for multiple different functional components as in conventional "all-in-one" nanomedicine design. The simple yet intrinsic multimodal nature of porphysomes represents a new "one-for-all" paradigm in nanophotonics design and also confers high clinical translation potential.

To discover new nanoscale photonic properties in porphyrin nanoparticles, we have created supramolecular assemblies of highly ordered porphyrin aggregates by mimicking the light harvesting systems found in nature, which possessed stimuli-responsive photonic properties (e.g., reversible/tunable photoacoustic sensing and self-regulating photothermal therapy).

To address the dark side of EPR, my lab has been exploring the use of light or sound to bypass or augument EPR to enhance nanoparticle delivery to tumors. For example, we designed a low molecular weight, targeted photosensitizer that can extravasate and homogeneously distribute within the tumor while binding specifically to cancer cells. When combined with laser light for sub-lethal PDT, this pre-treatment opens access to the deep layers of the tumor tissue for nanoparticle delivery without causing vascular shutdown. In addition, following our discovery of ultrasound-induced microbubble-to-nanoparticle conversion, we explored a strategy to harness this conversion as a tool to bypass the enhanced permeability and retention effect in order to directly deliver nanomedicines into tumors.

COURSE 7

Avigdor Scherz earned his BSc, MSc and PhD from the Hebrew University of Jerusalem in physics, chemistry, and biophysics, respectively. He did postdoctoral research at the University of Illinois in Champaign/Urbana and at the University of Washington in Seattle.Prof. Avigdor Scherz is a current Member of Scientific Advisory Board at Salio and Professor at the Department of Plant Sciences at the Weizmann Institute and Head of the Minerva-Avron Center for Photosynthesis. His research interests include photodynamic therapy, rational design, synthesis and application of new PDT reagents, physicochemical aspects of photosynthesis and biological redox reactions, photobiology and chlorophyll chemistry. He has published more than 160 papers and 16 book chapters. Prof. Scherz is also the co-holder of several patents that provided the groundwork for technology transfer and establishment of new start-up companies. He has 4540 citations and h-index 40.

Lecture1 –Radical production by TOOKAD and light; Type 1 PDT
Lecture 2- The tumor as an organ- A paradigm shift in photodynamic therpy
Lecture 3-Vascular targeted VTP: from bench to clinic and from local to systemic therapy.

COURSE 8

Laura M. Lechuga is a Professor and holds the position of CSIC Research Professor at the Catalan Institute of Nanoscience and Nanotechnology, in Spain, and head of the ICN2 Nanobiosensors and Bioanalytical Applications Group. Prof. Lechuga was an adjunct professor at the Artic University of Norway (department of Physics and Technology). She has also been a distinguished visiting professor at the School of Electrical and Computer Sciences of the *Universidade Estadual de Campinas* (Brazil) since 2013. Her research interests include the technological development of nanophotonic biosensors, their integration into portable lab-on-a-chip platforms and their application in clinical and environmental diagnostics. She has published more than 200 articles, book chapters and conferences, and more than 350 invited presentations. Prof. Lechuga is also a holder of 8 families of patents, co-founder of the technology company BIOD, associate editor of the IEEE Photonics and Optics and Laser Technology journals, and on the editorial board of the Journal of Sensors. She is a member of the International Society for Optics and Photonics (SPIE), the European Optical Society (EOS), the Spanish Optical Society and the *Real Sociedad Española de la Física* (RSEF). Member of advisory boards in Spain, Australia and Mexico, she received several prizes and recognitions over the years, including the 2016 Physics, Innovation and Technology Prize from the *RSEF* and the *Fundación BBVA*. Prof. Lechuga has 7453 citations, and an *h*-index of 47.

Photonic Biosensors for Clinical Applications

This lecture series will provide an overview of the different types of photonic biosensors, including plasmonics, nanoplasmonics and silicon photonics biosensors (as ring resonators or interferometers). The working principle, design, fabrication and performance of each type will be discussed. In addition, a description of the main biofunctionalization techniques routes will be explained as well their integration in full-compact lab-on-chip platforms to be used as point-of-care instruments. The crucial aspect of the applicability of biosensors devices for the early diagnosis of diseases (as cancer or infections) in real clinical environments will also be discussed to demonstrate their impact in Health and Society.

LESSON 1. General Introduction to photonic biosensors. Plasmonics and Nanoplasmonics biosensors. Biofunctionalization routes.
LESSON 2. Photonic biosensors based in silicon photonics. Lab-on-chip integration.
LESSON 3. Point-of-care platforms for Clinical Applications.

COURSE 9

Mauricio da Silva Baptista is a Full Professor and the Head of the Biochemistry Department on the Institute of Chemistry of the University of São Paulo. He's also an associated editor of the Photochemical and Photobiological Sciences journal, published by the Royal Society of Chemistry, is a member of the Scientific Reports editorial board and is a board member of the Brazilian Society of Biochemistry and Molecular Biology. His research interests include quantitative measurements in cells, membranes and interfaces, surface plasma resonance, protein-membrane interactions, photochemistry, photobiology, photodynamic therapy, redox processes, autophagy and mechanisms of regulated cell death. Prof. Baptista has published more than 190 papers (Web of Science), 12 book chapters, holds 7 patents and he has 5545 citations and h-index 43 (Google Scholar, march 7, 2019).

The role of contact dependent reactions in the efficiency of PDT

Photosensitized oxidations, which are reactions provoked by the interaction of light with photosensitizer (PS) molecules, are being used in medical technologies, such as photodynamic therapy, in order to trigger oxidation of biomolecules and consequently to eliminate cancer cells or pathogens. Damage in cytoplasmic or organelle membranes is key to modulate the mechanism as well as the overall efficiency of regulated cell death. There are two major mechanisms of photosensitized oxidations, called type I and type II, representing respectively, the direct oxidation of biological targets (direct-contact reactions) and the oxidations mediated by diffusing species, such as singlet oxygen. Nevertheless, the detailed molecular steps leading to biological injury remains largely uncharacterized and it is not clear how precise can be the spatial damage induced by the photosensitized oxidation reactions. In case of direct-contact reactions, the damage is performed precisely in the place where the excited species is generated and for type II processes, singlet oxygen or other diffusing species can carry oxidation potentials hundreds of nanometers or of micrometers away from the point of light absorption. In a recent publication, we demonstrated that for a PS to fully compromise membrane function, it needs to be sacrificed through contact-dependent reactions, forming lipid-truncated aldehydes, which are the active agents causing membrane leakage. Therefore, relevant damage that definitively changes the outcome of cells are precisely the locus of PS location, and therefore, justifies the search for molecular-specific oxidation-induced photodamage. Also, PS regeneration should be exploited as an effective tool to maximize the effects of photosensitized oxidations.

Increasing the efficiency of PDT by targetting Intracelullar organelles.

Photodynamic therapy (PDT) is a clinical modality used to treat cancer and infectious diseases. The main agent is the photosensitizer (PS), which is excited by light and converted to a triplet excited state. This latter species leads to the formation of singlet oxygen and radicals that oxidize biomolecules. The main motivation for this review is to suggest alternatives for achieving high-efficiency PDT protocols, by taking advantage of knowledge on the chemical and biological processes taking place during and after photosensitization. We defend that in order to obtain specific mechanisms of cell death and maximize PDT efficiency, PSes should oxidize specific molecular targets. We consider the role of subcellular localization, how PS photochemistry and photophysics can change according to its nanoenvironment, and how can all these trigger specific cell death mechanisms. We propose that in order to develop PSes that will cause a breakthrough enhancement in the efficiency of PDT, researchers should first consider tissue and intracellular localization, instead of trying to maximize singlet oxygen quantum yields in in vitro tests. In addition to this, we also indicate many open questions and challenges remaining in this field, hoping to encourage future research.

COURSE 10

David Boas is the Director of the Boston University Neurophotonics Center and is a Professor of Biomedical Engineering. He received his BS in Physics at Rensselear Polytechnic Institute and PhD in Physics at the University of Pennsylvania. His research interests include neurophotonics, Biomedical Optics, Oxygen delivery and consumption, Neuro-vascular coupling and Diffuse Optical tomography. He has also published more than 400 papers (Web of Science). Prof. Boas is also a holder of more than 20 patents. He is the founding President of the Society for Functional Near Infrared Spectroscopy

and founding Editor-in-Chief of the journal Neurophotonics published by SPIE. Prof. Boas was awarded the Britton Chance Award in Biomedical Optics in 2016 for his development of several novel, high-impact biomedical optical technologies in the neurosciences, as well as following through with impactful application studies, and fostering the widespread adoption of these technologies. He also a fellow of SPIE, AIMB and OSA. He has 34548 citations and h-index 105 (Google Scholar, march, 7 2019).

Neurophotonic Investigation of Cerebral Oxygen Delivery and Neurovascular Coupling

This course will cover several technological innovations from the last 10 years that have enabled us to study oxygen delivery to the brain with increased spatial and temporal resolution. These methods are being used for physiological and pathophysiological studies in human and animal models. Importantly, they have played a critical role in advancing our understanding of the vascular response to brain activity that serves as the basis for the ubiquitous fMRI BOLD signal that is revolutionizing the cognitive sciences. As such, a significant portion of this class will review the studies of neurovascular coupling and exploration of the basis of the fMRI BOLD signal.

The lectures will cover:

- Utilizing Speckle to Quantify Cerebral Blood Flow
- Neurovascular Coupling
- Exploring the Basis of the fMRI BOLD Signal

COURSE 11

Anderson Zanardi de Freitas had training in executive management and leadership by the Massachusetts Institute of Technology (MIT-Sloan program). He is currently a researcher and the manager of the Technology Innovation Center of IPEN. His research interest is in health care, with emphasis in methods for Optical Diagnosis. His main research areas are Optical Coherence Tomography application in dermatology, dentistry and cosmetology, and applications of femtosecond laser ablation of materials and X-ray generation. Prof. Freitas has more than 80 publications, including articles and book chapters. Dr. Freitas has 1259 citations and h-index of 20 (Google scholar).

Lucas Ramos De Pretto graduated in Systems Analysis and Development at the Technology School (Fatec) Baixada Santista - Rubens Lara in 2011, has a Master's degree in Nuclear and Energy Research Institute (IPEN) from the University of São Paulo in 2015. His main research area is Computer Science, with emphasis on Signal Processing. He also has experience in Computer Networks, having more than one-year exclusive training from SENAI in this area. He has also studied and programmed systems for mobile devices with the Android platform. Currently, he is a PhD student at IPEN and performed an internship at the Massachusetts Institute of Technology. His thesis is about the use of speckle patterns in optical coherence tomography images for characterization of microfluidic circuits and tissue microcirculation. De Pretto has 6 articles, 24 citations, and an h-index of 4.

Optical Coherence Tomography - Fundamentals and Applications

The theory behind low-coherence interferometry will be briefly described, in order to develop an intuition of how OCT works (and why it works). A few functional extensions of OCT will be discussed along some major applications in life sciences and industry-related fields. This will enable the audience to be familiar with the main OCT literature and come up with potential applications to their reserach field.

Speckle in OCT and OCT Angiography

We'll briefly discuss speckle noise and it's origins in OCT signal. The role of Speckle in OCT is important because, even though it degrades image quality, it is a useful source of information about moving scatterers inside a sample. This will lead up to a discussion on one of the main research topics in OCT literature, which is angiographic measurement/mapping. We'll review the major methods and algorithms proposed on the literature and discuss some interesting applications.

Invited Speakers

LECTURE 1

Denise Maria Zezell has Bachelor in Physics (1984), MSc in Physics (1987), PhD in Sciences from the University of Campinas (1991) and has specialization in Medical Physics in the International Center for Theoretical Physics-Trieste, Italy (1988 and 1990). She received her postdoctoral degree from the International Center for Theoretical Physics-Trieste, Italy (1992) and from the IPEN / CNEN-SP Lasers and Applications Center (1992-1995). She is a researcher at the Energy and Nuclear Research Institute of the National Nuclear Energy Commission since 1996, which she was Manager of the Lasers and Applications Center in 2008. Has experience in Physics, focusing on Optical and Spectroscopic Properties of biological tissues or material for health use, aiming the development of new diagnostic and therapeutic methods for clinical use, based on photonics. The main techniques used are micro-FTIR, ATR-FTIR, infrared thermography, high intensity lasers. She has published more than 173 papers with 1741 citations and h-index 27 (Web of Science). She also published 27 book chapters and holds 3 patents.

Clinical needs boosting Biophotonics developments

The talk will focus on different applications ranging from Lasers in Dentistry to spectroscopic methods, non-linear microscopy and spectral imaging for disease diagnosis such as hemangioma well as lung, thyroid and skin cancer.

LECTURE 2

Kleber T. Oliveira is Bachelor in Chemistry from University of São Paulo and obtained his PhD in Science in the area of Synthetic Organic Chemistry, in 2006, at the same University. He performed postdoctoral at the University of Aveiro, Portugal (2006-2007) and another at University of São Paulo (2007-2008), working on the synthesis of new photosensitizers. He also was Visiting Professor at the Department of Chemistry & Biochemistry of Florida State University in 2015. He has been Associate Professor at the Department of Chemistry of Federal University of São Carlos since 2010, and head the Laboratory of Bioorganic Chemistry. He has experience in the field of Organic Synthesis and acts in the development of new synthetic methodologies involving photocatalysis, photochemical reactions in continuous flow regime, development of continuous reactors and acts in the preparation of analytical standards for the pharmaceutical market. His research interests are the synthesis of photosensitizing molecules with potential biological, photosensitizing and photocatalytic activities with applications in Photonic Therapies and Organic Synthesis. He has published more than 78 papers (Web of Science), 1 book, and 4 book chapters. Prof. Oliveira is also a holder of 3 patents. He has 769 citations and h-index 15.

Machine Assisted Synthesis of Naturally Occurring Curcuminoids for Applications in PDT Treatments

Curcumin and its naturally occurring derivatives are present in turmeric (*Curcuma longa*) and are traditionally used as coloring in cooking. Recently, numerous applications of curcuminoids have been found in both medicinal chemistry and as photosensitizer in photodynamic therapy. Herein, it will be presented a developed approach for the machine assisted synthesis of curcumin and two others natural curcuminoids, all carried out by the same integrated protocol. A telescoped approach under continuous flow conditions was developed and compared to the combined flow–batch and all batch systems, demonstrating that the combined flow-batch approach is more cost competitive. Additionally, endeavors for the scaled-up synthesis of curcumin (kilogram-scale) is under development in order to allow a broad

application of curcumin as photosensitizer in *Aedes aegypti* larvae control using the photodynamic effect.

LECTURE 3

Anderson Rodrigues Lima Caires is associate Professor at the Federal University of Mato Grosso do Sul and Collegiate titular Member of the PostGraduate Program in materials science. His research interests include optical spectroscopy of materials, photodynamic inactivation of microorganisms, development of materials, methods for environmental assessment, Optical Characterization of Biological Systems and Biodiesel. He also has experience in atomic and molecular spectroscopy Magneto-Optical Trapping and collisions between cold atoms. He has published more than 50 papers, 37 monographs, and 4 book chapters. Prof. Caires is also a holder of 4 patents. He has 794 citations and h-index 14.

Chlorophyll fluorescence imaging for evaluation of metal and oxide-metal nanoparticles effects on photosynthetic activity of plants

Nanotechnology is an emerging field in science and engineering, which presents significant impacts on the economy, society and the environment. The nanoparticles' (NPs) production, use, and disposal is inevitable leading to their release into the atmosphere, water and soil where there are uncertainties about its fate, behaviour, and toxicity. Recent works have demonstrated that NPs can penetrate, translocate, and accumulate in plants. However, studies about the effects of the NPs on plants are still limited because most investigations are carried out in the initial stage of plant development. The understanding of the interactions between NPs and plants is fundamental because plants are the basis of food chains and essential in all ecosystems. In this context, it is aimed to show that chlorophyll fluorescence can be used as a non-destructive technique for studying the physiological state of adult plants submitted to the NPs, especially monitoring the photochemical efficiency of photosystem II (PSII).

LECTURE 4

Rickson Mesquita is an Assistant Professor at the Physics Institute "Gleb Wataghin" of the Campinas State University (UNICAMP). He majored in Physics and got his PhD at the same University. During his PhD, he worked as a visiting researcher at the Massachusetts General Hospital in Boston and after getting the degree he spent around 2 years as an associate researcher at the University of Pennsylvania in Philadelphia. His PhD thesis was a pioneer in Brazil for introducing optical diffusion methods to neurosciences as well as for integrating these methods with other multimodal methods such as MRI and fMRI. His research interests include light propagation in turbid or dense media applied to Biology and Medicine, with emphasis in optical diffusion and neurosciences instrumentation. The current areas of his interest include biomedical optics, light transportation in diffusive media, tissue optical properties, functional spectroscopy, photodynamic therapy and cancer.

Functional Near-Infrared Spectroscopy for Neuroscience

Functional near-infrared spectroscopy (fNIRS) employs photons in the near-infrared (~700-900 nm) to probe deep tissues continuously and noninvasively. The technique has been largely used in Neuroscience applications since it is sensitive to hemodynamic changes underlying neural function. In addition, the technique can be used in different scenarios (e.g., during exercise and in natural

environments) and with diverse populations (e.g., newborns) without risk. In this talk I will present the major developments of fNIRS in Neuroscience and discuss the state-of-the-art in this field. In particular, I will focus on our recent advances on methodology to better understand the brain at rest.

LECTURE 5

Luciano Bachmann is currently Associate Professor at the Faculty of Philosophy, Sciences and Letters of Ribeirão Preto (FFCLRP / USP), and is the coordinator of the extension project: Circus of Physics and Experimenting with Physics. His research activities consist of optical radiometry, including spectroradiometry, laser radiometry and its interaction with biological tissue looking for tissue dosimetry, effective irradiance and biological action spectra determination. His second research area are mainly vibrational and fluorescence spectroscopy applied to tissue diagnosis and biochemical characterization. He has published 68 papers, 1 book and 2 book chapters. He has 1667 citations and h-index 22.

Light dosimetry

Light dosimetry is crucial to improving procedures which involve photochemistry processes. Photon absorption by photoactive molecules produce chemical reactions that lead to biological effects and serve as the basis of photodynamic therapy. Other applications can include water treatment with photocatalysis and restorative processes with dental composite light curing in dentistry. We highlight here the importance of employing the spectroradiometric data from the source and the optical properties from the irradiated target in light dosimetry. Lasers, Light Emitting Diodes - LEDs, lamps and the Sun are sources conventionally used for such procedures. Spectral information is usually evaluated but not employed to determination the absorbed photons or the effective fluence delivered to the target. Also, the optical properties are usually considered in irradiation procedures to estimate the light-tissue interaction and guarantee that the photons are absorbed and promote the desired effects. However, these parameters are not considered to ponderate the spectral dependence of the source, or to calculate the optical penetration depth, the total absorbed photons or the final fluence delivered on the target. We understand that the considerations mentioned above are sometimes difficult to process *in vivo* procedures or in turbid media. However, we think that it is essential to consider such parameters to improve the knowledge about light-matter interaction in many practical cases.

LECTURE 6

Ana Gabriela Salvio received her degree in medicine in 1998, residency in Dermatology (2002) and her Ph.D. (2006) from São Paulo State University (UNESP). Currently, she is a dermatologist at Amaral Carvalho Foundation. She has experience in Dermatology, with emphasis in Oncology and Photodynamic Therapy. She has published 20 papers, 7 book chapters, and presents 97 citations.

PDT in Dermatology: The experience of Skin Department of Amaral Carvalho Cancer Hospital

Topical Photodynamic Therapy (PDT) is emerging as a useful treatment in dermatology. It has been shown to be an effective treatment of actinic keratosis (a pre malignant lesion), Bowen disease and basal cell carcinoma (BCC). Basal cell carcinoma is the most common non-melanoma skin cancer in fair-skinned people in the world. It represents the most frequent cancer in United States, Brazil, Australia and others countries. Although it has a low mortality, non-melanoma skin cancer has a high morbidity as result of multiple surgeries, which are the most used treatment for basal cell carcinoma. PDT is one of the non-invasive treatments options for BCC. Particularly for nodular BCC, PDT has a Strength of Recommendation A and a Quality of Evidence I. If precisely indicated, PDT can achieve almost the same results as surgery (the gold standard treatment for skin cancer) However, one of the

major challenges of the treatment of BCC through PDT is the local recurrence. Risk factors for recurrence include tumour diameter, location, and histological subtype. Some studies report differences in effectiveness according to the photosensitizer used. Most of the available studies about the efficacy of photodynamic therapy are focused on short-term results, thus requiring more evidence of long-term results.

LECTURE 7

Debora Marcondes Bastos Pereira Milori is a senior research at the Brazilian Agricultural Research Corporation (EMBRAPA) and coordinator of the Optics and Photonics Laboratory of Embrapa Instrumentation. Her research interests include developing methods, sensors and equipment for analyses of soil organic matter, as well as studies of nutrients and contaminants and plant diseases. Debora is a Physicist, graduated from the University of São Paulo and has Master and PhD degrees in Physics from the Institute of Physics of São Carlos (IFSC-USP). She has published more than 110 papers (Lattes, March 06, 2019) and has contributed to more than 20 book chapters. She has been awarded the Bernard Gross Award by the Institute of Physics and Chemistry of São Paulo (USP) in her graduation in 1984 and has been honored by more 12 awards throughout her scientific career, including the Vale-Capes Sustainability Award 2016 for the Best Master's Dissertation on Global Climate Change of the student Alfredo Augusto Pereira Xavier from IQSC-USP. Dr. Milori has more than 2800 citations and h-index 29 (Google Scholar, March 06, 2019) and is a holder of nine patents.

Laser-Induced Breakdown Spectroscopy (LIBS): applications in agriculture and environment

Laser-induced breakdown spectroscopy (LIBS) has become a prominent analytical technique in recent years for real-time characterization of many kinds of materials. The technique can detect any element in the periodic table, requires little or no sample preparation, takes only a few seconds per sample and has a low cost per sample compared to traditional techniques. For example, with proper calibration, LIBS also has great potential for real-time in-field soil analysis and precision farming. Essentially, LIBS is a multi-element analytical technique based on atomic spectroscopy that employs a high-energy laser pulse focused onto a sample surface to create a transient plasma. Emission lines from the plasma allow identification and quantification of all elements in the sample. LIBS has been successfully applied to quantifying many elements in different agri-enviromental matrixes, including soil carbon, plant nutrients, and heavy metals in landfill leachate. This talk will introduce basic concepts of LIBS and some applications in precision agriculture and environmental monitoring. Traditional and new techniques will be compared to LIBS along with their advantages and disadvantages.

Poster Sessions

SESSION 1 - 22 March, 2019 - 10AM

S1-01. *In vitro validation of snap-tag based antibody fusion proteins conjugated to near infrared photosensitizer on melanoma*

Nsole Biteghe F.A, Padayachee Eden, Barth Stefan South African Research Chair in Cancer Biotechnology, Department of Integrative Biomedical Science, Institute of Infectious Disease and Molecular Medicine, Faculty of Health Science University of Cape Town

An EGFR receptor targeting SNAP tag fusion protein was engineered for immunodiagnosis and treatment of EGFR expressing melanoma cells and its efficacy was compared to standard combinatorial effect of PDT and chemotherapy (Hypericin-PDT and DTIC) as an adjunctive combination therapy treatment to decrease cellular chemoresistance in melanoma. Single chain variable fragments (scFv) of panitumumab were cloned into a transient eukaryotic SNAP expression vector co-expressing cytosolic. After successive restriction enzyme digestion; ligation and transformation into bacteria, Plasmids were isolated and transfected into HEK293T cells using XtremeGene HP transfection reagent. Successful transfection was estimated by microscopic viewing of GFP expression. Zeocin treatment (100 ng/ml) was used to enrich transfected cells grown in enriched media. Supernatant containing secreted protein was collected every 3-4 days for a period of ±3 weeks. Supernatants were pooled and centrifuged to remove cells and purification was performed using immobilized metal affinity chromatography. Thereafter, successful purification of the recombinant protein was confirmed by SDS-PAGE and Western blot. Binding activity and cytotoxicity of the fluorescent-labelled and/or IR 700 conjugated 1711(scFv)-SNAP was documented on EGFR expressing melanoma cells using respectively flow cytometry, confocal microscopy and XTT assay. Lastly, cytotoxic activity, self-renewal capacity, ABCB5 and ABCG2 expressions after HYP-PDT+DTIC were determined using XTT, Clonogenic assays and flow cytometry. Cell viability results showed that, cell specific cytotoxicity of the scFv1711-SNAP-IR700 (nM), was superior to HYP-PDT + DTIC (in uM). Moreover, resistance to HYP+DTIC in DTIC resistant melanoma was correlating with an increased self-renewal capacity, ABCB5 and ABCG2 expression. This project describe a proof of concept of targeted phtoimmunotheranostics on EGFR expressing melanoma cell using SNAP-tag based PDT.

S1-02. Digital X-Ray Radiology Scheme Based On Compressive Sensing

Juan Pablo Hoyos Sanchez - Universidad Antonio Nariño, Vanderlei Salvador Bagnato - Universidade de São Paulo, Sebastian Pazos - Universidad Nacional de La Plata

Digital radiology is a widely used technique for generating digital images without the requirement of a radiologic plate. Among many of its applications has been widely used to study organs of living beings, but its main disadvantages lies in the necessary time of exposure to X-ray radiation to generate an image with good contrast, spatial resolution and low noise. The aim of this project is try to reduce the patient's level of radiation exposure by using fewer X-ray, similar to the proposed schemes in magnetic resonance imaging systems with compressed sensing. To develop the proposed scheme we must give answers two fundamental questions: How should the linear measurement process be designed?, to address this problem we will use the deterministic matrices theory so that the matrix design satisfies the Restricted Isometry Property – RIP and the condition of being highly incoherent. In addition, since this class of matrices has a low complexity, storage capacity and low implement cost compared with random matrices, will facilitate the development and implementation process. The second question is associated with how to design efficient algorithms to recovery the X image from measurement vector Y?, to solve we will exploit the image's structure and we will use proximal splitting methods as they offer tools to development easily implementable and scalable solver algorithms. Finally, we propose to study a scenario with impulsive noise, to model this noise present in the image we will use the theory of sparse Levy process because it exploits the sparsity present in real signals, and we will use this information into the solver to try to increase its performance.

S1-03. Optical and diffusion properties of diabetic tissues

Daria K. Tuchina,1,2,3 Polina A. Timoshina,1,2, Alla B. Bucharskaya,4 Valery V. Tuchin1,2,5
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Diabetes mellitus is one of the widespread worldwide diseases, the development of which disorders the functioning of vital organs of the organism. Therefore, investigation of the metabolic processes that occur in tissues at diabetes contributes to the development of disease diagnostic and treatment methods, as well as the prognosis of its complications. The goal of the study was to measure optical and diffusion parameters of rat tissues when alloxan diabetes develops.The spectra of diffuse reflection, total and collimated transmission of biological tissues ex vivo of white rats of the control and diabetic groups were measured. The recording of tissue samples spectra was carried out on a UV-3600 spectrophotometer (Shimadzu, Japan) in a wide spectral range of 350-2500 nm with a step width of 5 nm. Inverse Monte Carlo technique has been used for processing the experimentally measured spectra of the samples; wavelength dependence of absorption and scattering coefficients, and anisotropy factor has been obtained.Diffusion coefficients of chemical agents were quantified using collimated transmittance spectra of the ex vivo tissue samples taken from control and diabetic groups of white rats measured by the multichannel fiber-optic spectrometer USB4000-Vis-NIR (Ocean Optics, USA) in the spectral range of 500-900 nm during their immersion in agent solutions.For in vivo study of skin permeability for chemical agents in control and diabetic groups the optical coherence tomograph Spectral Radar OCT System OCP930SR 022 (Thorlabs Inc., USA) with a wavelength of 930 nm was applied.Studies were performed for skin, skeletal muscle, myocardium and some other tissues. Glycerol was tested for their permeability.As a result, we identified difference in optical and diffusion properties of diabetic tissues in comparison with healthy tissues both ex vivo and in vivo. We have found that the diffusion of chemical agents is much slower under the conditions of alloxan-induced diabetes.

S1-04. Optical non-invasive diagnostics of microcirculatory disorders in patients with diabetes

Elena Zharkikh, Victor Dremin, Elena Potapova and Andrey Dunaev (Research and Development Center of Biomedical Photonics, Orel State University named after I.S. Turgenev, Orel, Russia)

The problem of diabetes (DM) has attracted scientists of different specialties, as its prevalence is increasing worldwide and assumes the character of a pandemic. Recent studies have noted that timely diagnosis and treatment, including increasing patient control, reduces complications of DM and makes it possible to reverse their development if detected in the early preclinical stages. The aim of this work was to analyze the possibilities of the laser Doppler flowmetry (LDF) in assessing microhaemodynamics changes in the feet of patients with DM at the early stages.The experimental studies were performed using laser multifunctional complex "LAZMA-ST", that realizes LDF with a probing wavelength of 1064 nm. Local heating tests with different temperature modes were used to assess dynamic changes in skin perfusion. To provide thermal effects the device "LAZMA-TEST" was used. The study involved 76 patients with DM and 47 healthy volunteers. Wavelet analysis of registered LDF signals was performed to study underlying physiological mechanisms associated with various blood flow regulatory systems.The tissue perfusion was slightly higher in diabetic patients comparison to controls without statistically significant difference under basal conditions. Heating provoked significant vasodilation in all subjects, but its rate was statistically smaller for patients. That indicates insufficient regulation of blood microcirculation system by mechanisms that provide vasodilation. A reduced amplitude of cardiac oscillations was also detected in patients with DM.Thus, the hypothesis about the possibility of assessing microcirculatory disorders with the use of spectral analysis of LDF-records has been verified. A promising direction for further research is the implementing of proposed diagnostic method to other socially significant diseases. This will allow development of a new non-invasive diagnostic method for identifying microcirculation disorders at earlier stages.

S1-05. Pre-clinical validation of transrectal diffuse optical tomography for monitoring photocoagulation progression during photothermal therapy

Celina L. Li1,2, Carl J. Fisher2, Runjie Bill Shi2,3, Robert A. Weersink2,4, Jie He2, Gang Zheng1,2, and Brian C. Wilson1,2
1 Department of Medical Biophysics, University of Toronto, Toronto, Canada
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Background: Diffuse optical tomography in a transrectal configuration (TR-DOT) has been developed to monitor progression of the photocoagulation front during interstitial photothermal therapy (PTT) of focal prostate cancer. Based on feasibility studies in phantoms and ex vivo tissues, we have been testing the DOT system in preclinical normal and tumor-bearing canine prostate models with/without porphysome (PS) infusions. Methods: Co-registration of DOT measurements with magnetic resonance imaging (MRI)-based thermometry in photocoagulating phantoms were performed to provide temperature profiles for correlation with DOT signals changes. TR-DOT measurements of prostate are collected under anesthesia at probing wavelength 670nm, 750nm, and 808nm to assess signal stability. Device responsivity to different PTT protocols are evaluated by collecting DOT measurements during treatment delivery in healthy canine models on prostate, kidney, muscle, and lung. Correlation between relative DOT signal changes and lesion sizes are attempted by comparing with histology result of photocoagulation zone. DOT measurements of in vivo tissue optical properties are collected to ascertain its sensitivity and provide input parameters for PTT simulation. Results: Preliminary results have shown that the DOT system can detect blood content and PS uptake by measuring different absorption coefficients at the three probing wavelengths. DOT signal stability is found to be variable individually. Pulsatile signal fluctuation up to 40% as well as stable signal fluctuating within 10% are both observed, which is potential owing to differential vasculature at the detection site. The device can show relative signal change in 30 seconds into PTT, confirming its sensitivity. Conclusion: DOT instrument as a minimally-invasive technique to monitor PTT is feasible. Further investigation is needed to address current confounding issues. This work is supported by the Terry Fox Research Institute (grant #1075).

S1-06. Study on mid-IR spectroscopy of glucose-insulin and blood samples for non-invasive glucose quantification.

Gerardo Romo-Cardenas, J. de Dios Sánchez, Juan I. Nieto, Claudia Gómez, (UABC), Santiago Camacho (CICESE), Maria Cosio (UPP)

For the proper control of diabetes mellitus, it is necessary to carry out an adequate monitoring of the blood glucose concentration. Given the pain and discomfort associated with the digital glucometer, various efforts have been made in order to develop non-invasive optical techniques for glucose measurement. Within these techniques, medium infrared spectroscopy stands out. Where, although the results show to be favorable, they remain partially satisfactory due to the lack of interdisciplinary approaches. In particular, with respect to the compounds that participate in the mechanism of glucose regulation that could participate in the spectroscopic measurements. This leads to propose a strategy of interdisciplinary approach in order to analyze in detail the interaction between light, glucose and compounds that participate in its regulatory mechanism in order to know the effect of these substances on absorption spectra and possible ranges of the spectroscopy techniques for the measurement of glycemia. The results of this investigation show that glucose and insulin do indeed overlap in the spectral region in the region of 1000cm-1. Likewise, the use of the Nyquist theorem makes the pre-processing of bio-optical signals more efficient. In such a way that when applied within a factorial experiment design, it was found that glucose has a greater participation in the absorption spectrum. This experimental design also helped to find, despite the spectral overlap, that there are no structural changes in the samples due to the presence of both compounds, which have an effect on the absorption spectrum.

S1-07. Construction and evaluation of a frequency-domain diffuse optical spectroscopy device for brain monitoring in the clinic

Rodrigo Menezes Forti (UNICAMP), Rickson Coelho Mesquita (UNICAMP)

Diffuse optical spectroscopy (DOS) aims to investigate tissue physiology millimeters to centimeters below the tissue surface. The technique has made significant progress in the past few decades, and it is currently an established imaging technique to probe tissue physiology, particularly in the brain. Despite its potential, DOS has yet not made significant impact as a continuous, bedside monitoring tool in the clinic. The goal of this project is to construct and characterize a portable frequency-domain DOS instrument to provide absolute oxygenation measurements in the clinical setting, in real time. The proposed instrument will be employed in a clinical setting along with a diffuse correlation spectroscopy instrument to provide real time oxygen metabolism in the brain, and its usefulness will be evaluated in the neuro-intensive care unit with traumatic brain injury patients.

S1.08 - Analysis of the influence of physical activity in the decision-making process from optical diffusion techniques

G.G. Martins (IFGW), A.F. Quiroga (IFGW) and R.C. Mesquita (IFGW)

The biological reason for a physical exercise shutdown is still subject of debate. The literature provide two potential explanations for the phenomenon, related to either the result of muscular exhaustion or the outcome of an intrinsic and unconscious decision made by the subject. The question emerged after the observation that the cerebral oxygen levels, especially in the prefrontal cortex region, decline just before the activity shutdown, suggesting that the end of the exercise may be due to a decision making process. This topic has been of interest due to the potential use of neuroimaging techniques to better train cognitive skills related to exercise, providing a better performance. The present research project aims to develop an experimental protocol that enables simultaneous measurements of muscular and cerebral oxygenation. With this purpose, physically active subjects were submitted to strength tests using dumbbells at different intensities, while near-infrared spectroscopy were used to measure brain and muscle oxygenation throughout the activity. In the analysis, we remove motion artifacts from the collected data and decontaminate brain signal from influences of systemic physiology. At last, we compare brain and muscle oxygenation in order to analyze the trigger to stop physical activity.

S1-09. Computational Photo-Scatterography

Melissa White and Alyosha Molnar - Cornell University

Biomedical imaging of tissue using scattered photons suffers a fundamental trade-off between depth and spatial resolution due to photon scattering. Visible to infrared wavelengths of light are ideal for in vivo tissue imaging because they penetrate deep into the body, are safe and non-ionizing, inexpensive, and easy to control. However, photons in these wavelengths are scattered by tissue hundreds of times before detection, resulting in an extremely low SNR.Our proposed method introduces analog sensors never before used in tissue imaging applications. Angle-sensitive pixels (ASPs) are able to register the angle of incident light, which will take on different statistical distributions depending upon depth, scattering coefficients, and absorption coefficients of the medium. With an a priori model of how photons interact in tissue, we can predict which photon paths are more likely, and reduce the SNR by simultaneous angle-gating and location-gating of backscattered photons.One issue with image detectors that need to be sensitive to photon count is that they saturate, and additional collected photons will not be registered. In applications with excess background, this is a particular problem. We design the pixels in conjugate pairs – with responses sensitive to +/- theta. A differential amplifier is used to reject the common mode voltage between the two pixels and reset using the voltage difference. Further, two reset switches are connected in series, which can be used to reset different parts of the sensor chip at different rates. Thus we have a flexible programmable sensor where hardware parameters can be designed in conjunction with software. Methods being explored for this device include lensless interferometric imaging of backscattered light; reflection-mode Fourier ptychography; and angle-gating combined with machine learning techniques to extract richer information from small amounts of backscattered light.

S1-10. Mediating role of cortical responses to maternal affective touch in the relationship between maternal sensitivity and emotional regulation: an fNIRS study

Camila Junqueira Muylaert - Mackenzie Presbyterian University

Emotion regulation regards the processes involved in the management and modulation of emotional activation, being associated with positive development, lower rates of behavioral problems and adaptive behavior. Ample evidence suggests that maternal sensitivity, the ability to perceive and interpret the infant's cues, and to respond in a prompt and appropriate manner, contributes significantly to infant emotion regulation. On the other hand, affective touch is also considered by some researchers as promoting early emotional regulation. However, the possible mediating role of cortical responses to maternal touch in the relationship between maternal sensitivity and infant emotion regulation remains unexplored. A relatively recent neuroimaging technique, Near Infrared Functional Spectroscopy (fNIRS), has been promising in assessing the cortical response patterns of infants. This project aims to analyze the predictive role of: a) maternal sensitivity to the infant's emotion regulation; b) maternal sensitivity to the infant's cortical responses to maternal affective touch; c) the infant's cortical responses to affective touch to their emotion regulation. More importantly, we will test a partial mediation model, to explore whether the influence of maternal sensitivity to infant emotion regulation may (partially) occur via the infant's cortical responses to affective touch by the mother. To do so, we will evaluate maternal sensitivity at 6 months (N = 30 infants) applying the Ainsworth scales to a mother-infant interaction. At 10 months, cortical activations in response to maternal affective touch will be measured by fNIRS, targeting the frontal and temporal regions. At 12 months, emotion regulation will be assessed from child responses to episodes of negative emotions generated in a Laboratory setting.

S1-11. Method for the optical diagnosis of inflammatory diseases of the paranasal sinuses

E.O. Bryanskaya 1, I.N. Makovik 1,2, O.A. Bibikova 2, A.V. Dunaev 1,
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2 art photonics GmbH, Berlin, Germany;
3 Charité – Berlin University of Medicine, Berlin, Germany;

Diagnosis of inflammatory diseases of the paranasal sinuses is one of the urgent problems of modern otolaryngology. To date, radiography, computed tomography, magnetic resonance imaging, rhinoscopy and ultrasound are used to identify these pathologies. However, these methods have a number of drawbacks, which consist in the need to use roentgen radiation that makes it impossible to diagnose of pregnant women and children, a high level of false-negative results and in some cases, painfulness of the diagnostic procedure.To overcome these shortcomings, the application of the digital diaphanoscopy method seems to be promising. This method consists in translucence of the paranasal sinuses with low-intensity radiation of the visible and near IR ranges and visualizing the pattern of its scattering. Conducted preliminary studies using an experimental setup designed and assembled by art photonics GmbH (Germany) in collaboration with Charité – Berlin University of Medicine (Germany) revealed a high impact on the result of visualization of external illumination of the area under study. To minimize the influence of this factor, a protective screen was designed to exclude artifacts caused by external illumination.To identify the range of exposure values of the CCD camera to obtain maximum sensitivity to identify of pathological changes, experimental studies were conducted on healthy volunteers. The results of study showed variations in the scattering patterns of different volunteers with the same exposure value. This can be explained by such anatomic features as the structure of the skin, the thickness of the skull bone tissue, the size of the sinuses and their asymmetry.Taking into account the identified features, it is planned to conduct experimental studies with the participation of healthy volunteers and patients with inflammatory diseases of the paranasal sinuses and with subsequent comparison of the results of digital diaphanoscopy and magnetic resonance imaging.

S1-12. Multispectral label-free lifetime fluorescence imaging to distinguish skin lesions

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Skin lesions are the most common human cancer diseases, usually, is it diagnosed by clinical visual inspections followed by biopsy. Early detection of these diseases is critical, depending on an accurate and trained dermatologist and can increase the survival rate. Aiming for screening and early diagnose skin lesions many techniques are presented, however, optical techniques are highlighted since they are fast and noninvasive. In this context, fluorescence steady-state and lifetime imaging show potential by being able to image metabolic changes using endogenous contrast. Here it is demonstrated an in vivo label-free multispectral fluorescence lifetime imaging system to distinguish between two types of clinically similar lesions. A pulsed Nd:YAG laser emitting at 355 nm is used to excite the endogenous fluorophores and three channels of acquisition bands are used to imaging the skin. Preliminary results showed differences in the fluorescence lifetime between malignant and benign skin lesions. As well as the lesion and the healthy skin around, demonstrating a potential tool to identify the lesion and it edges. Machine learning methods were applied to clinically similar skin lesions to distinguish between malignant and benign, showing the potential of fluorescence lifetime to be applied as a screening technique.

S1-13. Early Detection of Oral Epithelial Cancer with Endogenous Fluorescence Lifetime Imaging Endoscopy

Elvis Duran(1), Dae Yon Hwang(1), Shuna Cheng(1), Rodrigo Cuenca(1), Bilal Malik(1), Beena Ahmed(2), Kristen C. Maitland(1), John Wright(3),Y. S. Lisa Cheng(3), Terry Reese(3), and Javier A. Jo(1)
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Increased metabolic activity, a hallmark of epithelial cell malignant transformation, induces subtle changes in the oral tissue autofluorescence. The optical “redox-ratio”, typically defined as the autofluorescence intensity of NADH/FAD, is sensitive to changes in the cellular metabolic rate. A decrease in the redox-ratio indicates increased cellular metabolic activity, as is typically observed in malignant cells. Specific changes in the fluorescence lifetimes of both NADH and FAD have also been associated with increased metabolic activity in malignant oral epithelial cells. We therefore hypothesized that more specific biomarkers of oral cancer and dysplasia can more accurately be quantified by endogenous fluorescence lifetime imaging (FLIM). In this work, FLIM images of benign, dysplastic and early stage cancerous oral lesions from 74 patients were acquired at three emission channels (390±20nm, 452±22.5nm and >500nm) using a handheld multispectral FLIM endoscope. For each pixel, the fluorescence decays collected at the three emission bands were analyzed using a biexponential decay model, resulting on 16 FLIM-derived parameters per pixel. Statistical analysis was performed on each of the computed FLIM parameters (paired t-test: Normal vs. Benign, Normal vs. Dysplasia/Cancer; t-test: Benign vs. Dysplasia/Cancer). Results from this analysis revealed that FLIM-derived parameters associated to collagen fluorescence, FAD fluorescence, and the optical redox ratio were statistically different ($P < 0.05$) in dysplastic/cancerous vs. benign oral lesions. These parameters were then used to train and cross-validate a Quadratic Discriminant Analysis (QDA) classifier for the discrimination of dysplasia/cancer from benign oral lesions. Classification performance was quantified using a Leave-One Patient-Out (LOPO) cross-validation approach, yielding levels of sensitivity >90% and specificity >85%, and Area Under the Receiving Operating Curve (ROC-AUC) >0.9.

S1-14. A clinically compatible handheld fluorescence lifetime imaging endoscope for label-free metabolic imaging

Oscar Benavides, Michael Serafino, Shuna Cheng, Javier Jo
Department of Biomedical Engineering, Texas A&M University, College Station, TX

We present our 2nd generation compact handheld simultaneous multispectral frequency-domain FLIM endoscopic system for label-free metabolic imaging of oral cancer, with enhanced optical performance and

system usability. Our custom-designed and 3D-printed handheld endoscope consists of an enclosure (6 x 3 x 3 cm3) with a rigid probe (1 cm diameter, 9 cm length) that weighs less than 125 g with all the system components, which, compared to our previous system, is significantly smaller and lighter, and has improved ergonomics and usability. The enclosure has mounts for a dual axes bi-directional MEMS scanner and a dichroic mirror, and plug-and-play ports for excitation, emission collection and rigid probe optics. The rigid probe used for oral mucosa imaging contains a three-lens imaging system that, compared to our previous system, has: an increased field of view (FOV) (6 x 6 mm2 vs. 16 x 16 mm2), improved lateral resolution (36 μm in the center and 65 μm at the edge, diffraction-limited performance across a central ± 5.5 mm field), and an extended working distance (10 mm vs. 40 mm). A 375 nm CW laser is used as the excitation source and is modulated at 1.25 MHz and 20 MHz, and scanned with a 5 mm MEMS mirror. The fluorescence emission is spectrally divided into three emission bands (405±10 nm, 440±20 nm, and 525±25 nm) targeting collagen, NADH, and FAD, which are relevant for early detection of oral cancer, and detected by three independent APDs. An FPGA is used to further process the multispectral signals and fluorescence lifetimes are estimated by computing the phase shift and demodulation at 1.25 MHz, 20 MHz and its harmonic frequencies up to 100 MHz via the Discrete Fourier Transform. Our clinically compatible handheld endoscope allows for noninvasive and fast in situ clinical metabolic imaging of the oral mucosa.

S1-15. Non-invasive optical determination of capillary refill time

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Non-invasive methods for blood perfusion monitoring are of extreme clinical importance such as in intensive therapy and anesthesia. Existing approaches include the use of finger pressure for subjective observation of skin color change, a surrogate for capillary refill time (CRT), and contact optical methods such as oximetry. To overcome these limitations, we are developing a quantitativeminimal contact CRT meter, using remote plethysmography (rPPG). In our proposal, we record skin relaxation via RGB video channels of video of a region of interest (ROI) to determine CRT after ROIskin is lightly pressed. Finger PPG is simultaneously recorded. We seek to understand how the implied CRT and PPG signals relate to age, skin types, blood pressure, and heart rate. Blood pressure is modified with a sphygmomanometer cuff with occlusion up to 50% of the volunteer's diastolic pressure, for 30 s. In this first stage of the study, 20 subjects ages 20 to 70 years old have served as volunteers. The ROI is approximately 4 cm2 of the volunteer’s inner forearm. Preliminary results have shown a consistent exponential decay of the green channel intensity, caused by the change of skin color during the relaxation after the skin has been lightly pressed. The relaxation time constant is the figure of interest. Further relationships are being investigated. In the second phase, we will have up to 100 volunteers for further validation and statistical analysis. We are working to develop a robust metric to determine small variations in the CRT, reflecting underlying physiological changes. We are also determining the minimum pressure that could be applied to determined CRT, to apply camera CRT for patients with sensitive skin. Finally, we are studying whether such CRT measurements and simultaneous finger PPG are complementary or redundant measurements. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

S1-16. Iridescent phenomena in Morpho cypris and Greta oto butterfly wings. A physics point view

C. P. Barrera-Patiño1, José Dirceu Vollet Filho2, Ramon Gabriel Teixeira Rosa2, A., Natalia Inada2, Vanderlei Salvador Vagnato2, R. R. Rey-González1
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In this work, we present a theoretical and experimental study of photonic properties of biological materials present in Colombian wildlife species, such as the wings of Greta oto and Morpho cypris butterflies. These systems present iridescent effects, which are related to the structures present on the surface of the materials of interest, it is characteristic of the structural colors. The theoretical modeling of the system allows to compare the photonic

band structure with optical properties of this systems. The effect of the inherent disorder that exposes these materials is analyzed in the context of Anderson's location. The implementation of Anderson localization in the study of photonic structures let to us identify the properties that disorder could introduce in these systems. Our results focus on the understanding of iridescence and the effect of disorder in photonic structures. This is a recent research field, in which every contribution allows its knowledge and subsequent implementation of photonics materials in technologies that enable human and industrial development.

S1-17. Clutter artifact reduction in photoacoustic imaging

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Introduction. Photoacoustic imaging (PAI) is a technique used to obtain functional information at molecular levels of biological tissue with simultaneous high optical contrast and high spatial resolution. On PAI, the energy deposited on tissue by pulsed light causes a thermoelastic expansion and an initial pressure rise, creating ultrasound waves. These waves can be detected by an ultrasound (US) transducer to generate PA images. In-vivo PAI is usually performed by illuminating the tissue from the same side where the US transducer is positioned to record the pressure waves. This geometry allows the acquisition of US and PA images simultaneously but, due to the high light absorption by melanin present on human skin, the PA waves generated at skin surface propagates into the tissue and are backscattered by structures presented on image plane, creating the clutter artifact, resulting in poor contrast, signal-to-noise ratio (SNR) and significantly limits imaging depth. In this study, methods to minimize the clutter artifact in PAI from a tissue-mimicking phantom containing a light absorber layer at its surface (simulating the skin) were investigated.Methods. For PA signal generation, Nd:YAG laser (Brio Quantel) was used delivering pulsed light at 532 nm wavelength with 5 ns pulse duration and 20 Hz repetition rate at a tissue-mimicking phantom manufactured using the copolymer styrene-ethylene/butylene-styrene (SEBS) in mineral oil. The signal was recorded with a linear US transducer (L14-5/38 Ultrasonix). To remove clutter artifact in PAI, an algorithm to compute the short lag spatial coherence (SLSC) metric was developed in MATLAB. Results. The SLSC PAI showed reduction of background signal with almost total elimination of the clutter artifact compared to conventional PA image. SNR for conventional and SLSC PA images was 2.16 dB and 3.91 dB, respectively. The results demonstrate that SLSC metric presents high potential to be used on clutter reduction in PAI.

S1-18. Measurement of optical properties with double integrating sphere

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Because of the diverse applications of optical radiation in medicine, increases the description of light propagation in biological tissues; and the optical properties determination. Characterizing its optical properties means determining the absorption coefficients (μa), scattering coefficient (μs) and the anisotropy coefficient (g). In this work, we employed two integrating spheres associated with Inverse adding-doubling (IAD) algorithm that calculates the values of μa, μs and g from the measured values of reflectance and transmittance. With this system we measured the coefficients of polyurethane phantom at the wavelength of 632nm.The experimental setup is composed by two integrating spheres model UMBB-150 (Gigahertz Optik, Türkenfeld, Germany) that have an internal diameter of 150 mm, an entrance aperture of 25.4 mm and coupling aperture of 37.7 mm, with internal coating of barium sulphate (~ 97% reflectance). Signal detection was performed by four silicon photodiode, (SFH 206K, Farnell) that was connected an Arduino plate (Arduino UNO R3) and monitored and stored at computer. The experimental measurements input to the IAD algorithm are the diffuse reflectance, diffuse transmittance, unscattered transmittance. We use this experimental setup to able to monitor possible beam fluctuations and normalize the measurements, furthermore, it makes the measurement of the reflection and transmission diffuses simultaneously reducing experimental errors. Our desire, at this stage of implementation is to achieve a reproducible and precise system. At this point we are now improving the method of data acquisition and edge of the electronic components. With this method we intend to determine the coefficients for a wide

spectral region by changing the lasers wavelength over the visible and infrared range. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001

S1-19. Dynamics of trapped particles in optical tweezers

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Among the Nobel laureates in 2018, for groundbreaking inventions in the field of laser physics, Arthur Ashkin was awarded for the creation of optical tweezers and their application to biology. A system of particles trapping consists in an objective with high numerical aperture, responsible for focus a laser beam, and a dielectric particles sample. When these approximate to the focus, the momentum exchange, due the incident photons scattering, generates a force that divides into two components: radiation pressure and gradient force. The contribution of both, is responsible for trapping particles in that region. The optical force is of the order of piconewtons, then micrometric or smaller particles can be manipulated. So the applications in biological sciences ranges from single molecule studies to cell biophysics. The objective of the present study is analyses the dynamic motion of micrometers particles trapped in harmonical potentials and calculate the trap stiffness of the optical tweezers. The calibration methods used provides results consistent with the literature and used parameters. In addition to studies with dynamically controlled optical potentials using AOM, we also intend to study nanoparticles samples, which can be trapped in the that system. Especially nanodiamonds with nitrogen-vacancy center. As a sensor, it is advantageous to be able to position the center in space and record electromagnetic or temperature information.

S1-20. Investigating radiation response of pancreatic tumors, their vasculature and microenvironment using in vivo optical imaging to identify new treatment strategies

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Despite therapeutic advances, the 5-year survival rate for locally advanced pancreatic cancer (PC) is ~4%. Recently, stereotactic body radiation therapy (SBRT) of PC is being explored clinically to maximize therapeutic efficacy by delivering a large and precise radiation dose to the tumor with minimal toxicity to surrounding tissues. However, we and others have shown that high-dose SBRT induces acute, yet transient vascular damage, increasing tumor hypoxia and, subsequently, the expression of hypoxia-inducible factor-1α (HIF-1α). As a result, tumor survival is promoted and metastasis is induced, leading to treatment resistance. Using a novel optical intravital imaging platform and orthotopic mouse model of a multi-reporter human PC cell line (BxPC3), we aim to investigate which SBRT-induced changes in the tumor microenvironment (TME: vasculature, stroma, immune cells) lead to tumor hypoxia and whether we can address tumor hypoxia by decreasing the tumor's O2 consumption. This can be achieved by using a HIF-1α inhibitor, such as metformin. With a transparent window chamber placed over the pancreas, we were able to non-invasively and longitudinally image orthotopic PC (DsRed-BxPC3 cells containing a GFP-labeled hypoxia response element) and its TME simultaneously at cellular resolution using in vivo fluorescence microscopy (Zeiss LSM710). Imaging was performed before (D0) and after (D1,D4,D7,D14) fractionated, high-dose SBRT (5x8Gy) with (n=3) or without (n=2) the addition of metformin. The level of tumor hypoxia was quantified as the ratio of GFP/DsRed intensities. Preliminary results support the hypothesis that metformin has the potential to decrease SBRT-induced tumor hypoxia. To confirm this, we are currently increasing sample size and performing immunohistological validation. This ongoing research will address key areas of hypoxia-associated treatment failure in PC and could potentiate the design of a new pilot clinical study in PC combining SBRT and metformin.

S1-21. Digital Holographic Microscopy and Tomography as a tool for quantification of cell mitosis

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Digital Holographic Microscopy (DHM) and Holographic based Tomography (HT) [1] provide possibility of label-free and in principle quantitative imaging of phase micro objects, which can be used in digital phase histopathology, single cell research or drug testing. DHM is very easy to apply for measurements and monitoring of transmission microstructure, however it delivers integrated information about optical path. To overcome this

problem in previous studies an approximation of the refractive index (RI) using a sphere shape or the method of changing the medium had been implemented with limited applicability. HT enables reconstruction of both 3D geometrical information and 3D distribution of RI in a single cell, including morphology of subcellular structures and local concentration of protein inside a cell. This in turn is closely connected with a dry mass of a cell. However HT measurement requires capturing several holograms and cannot be easily implemented in long term monitoring of living cells. In the paper I present the measurement strategy with the goal to quantitatively investigate the process of cell mitosis. We propose to connect time-lapse cells measurements performed at DHM during their growth with a few tomographic measurements, which deliver true 3D refractive index distribution in living cells. These will be used to decouple the refractive index and thickness information from a singleframe holographic acquisition. In the work I use the DHM and HT developed at the Institute of Micromechanics and Photonics, WUT. DHM is based on Mach Zehnder interferometer configuration with in-plane holograms capture and Fourtier transform method of phase reconstruction. The results of implementation of the proposed measurement startegy will be presented in the form of a complete description of the HaCaT keratinocytes mitosis process.

S1-22. See Below the Skin

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Current clinical practice for cellular-scale imaging is invasive. Diagnosis process may involve either a blood test which requires a blood sample or a biopsy test where a portion of the tissue is cut off for examination. Several non-invasive techniques exist like nuclear imaging, but often these techniques use ionizing radiation or nuclear substances which can damage the healthy cells. An alternative solution is to use light around the visible wavelength spectrum. Light travels deep inside the body. A simple experiment with a smartphone flash shone across the finger demonstrates the fact that a considerable amount of light can traverse deep into the body. The main advantage of light is its non-ionizing (400-1100nm) nature and is cheap to produce, control and sense. However, the challenge is that most of the pass-through photons are scattered, with an average of 10 scattering events per mm. By 50mm, a photon could face avg 500 scattering events! The result is that if we wanted to use light for imaging, we have a very large-scale inverse problem with low SNR. We wish to use techniques from computational photo-scatterography to solve large-scale bio-imaging inverse problems. The new computational imaging paradigm will jointly leverage all computing domains – optical, analog & digital, producing platform technologies to impact a host of medical and wellness applications, ranging from wearables to non-invasive point-of-care devices.

S1-23. Lensfree holography microscope to semitransparent samples imaging with digital processing on Python

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Due to the great importance of microscopic images and the current demand for simple and portable devices, lensfree microscopy is emerging as a great image technique. Compared to the traditional microscopy, this novel one has the greatest attractive of decouple field of view from image resolution. Our version of this microscope is an in-line holographic one, which has a field of view of approximately of 30 mm2 and 3 μm of resolution. The acquired images (holograms) are digital processing using Python Software Foundation (Python Language Reference. Available at http://www.python.org).

S1-24. Broadband system for optical properties determination of turbid media

Luismar Barbosa da Cruz Junior, Luciano Bachmann (FFCLRP/USP)

Introduced by Prahl in 1993, the IAD method (Inverse Adding double) is a tool to calculate the scattering (μs) and absorption (μa) coefficient. It's an algorithm based on numerical solution of transport equation, and uses reflected and transmitted light due integrated sphere. We assembled an integrating sphere in our laboratory in order to

employ IAD and acquire scattering and absorption coefficients over a broad spectral range, visible and near infrared. For the moment we describe the system for the visible only. The equipment was designed using Inventors Autodesk 2016 software and printed on a 3D printer at our department using PLA (Poly Lactic Acid) material. Two integrating spheres were used, with internal diameter of 150 mm, laser input and sample coupling of 25 mm, and internal coating of BaSO4. The source used for diffuse spectroscopy is a halogen Xenon 500W lamp (model 66921, Newport, Oriel Instruments U.S.A.). Delivery of light from the source and collection of reflected and transmitted intensities is carried out through a fiber optic system (QP600-2-SR, UV / SR-VIS High OH content 200-1100 nm, 600 μm, Ocean Optics). The diffuse reflected and transmitted radiation was captured by a spectrometer (USB4000, Ocean Optics). To validate the instrumentation, it was used polyurethane phantoms with TiO2 and india ink, with a nominal thickness of 6 mm. The results obtained in the characterization through the IAD algorithm (in duplicate) at 633 nm wavelength were μa = 0,000 ± 0,000 mm-1 and μs = 0,057 ± 0,003 mm-1 and demonstrates a great divergence of results to the reference values of μa = 0,001 mm-1 and μs = 0,477 mm-1. We observe a great divergence, near of one magnitude order, the results indicates that the equipment developed in our laboratory can be able to obtain the optical properties of the samples as proposed, but before the system must to be better characterized to evaluate radiation lost, reduce noise and after that acquire results from a calibrate phantom.

S1-25. Polarized light imaging of human breast cancer

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Polarimetry is a promising optical method to rapidly assess biophysical characteristics of tissues. Tissue properties are contained within the Mueller matrix (MM), a mathematical description of its interaction with polarized light. Biophysical quantities derived from the MM, such as depolarization and linear retardance can help differentiate tumor from surrounding healthy tissue. Our objective is to use polarimetry images and machine learning to automatically differentiate invasive breast cancer from normal surrounding tissue. A rapid and accurate intra-operative tool could help reduce re-operations needed to address inadequate surgical margins in breast conserving surgery (currently ~20%). Unstained slides (n=4) containing human invasive ductal carcinoma and surrounding tissue were imaged with a Mueller matrix polarimetry system to obtain depolarization, linear retardance, diattenuation, and cross-polarization images. Features for classification were measured independently on 200um x 200um regions of interests (ROIs, ~57,000 in total) into which each image was subdivided. Statistical features were extracted from all polarimetry ROIs, which were then classified using a random forest algorithm with leave-one-patient-out cross validation. The classifier was able to identify tumor with a sensitivity and specificity of 78% and 83% respectively. We anticipate improved performance with a larger dataset, as the classifier will generalize better. Depending on clinical application, the classifier can also be adjusted to improve sensitivity at the expense of specificity or vise-versa. The encouraging initial classification accuracy of breast cancer slides with polarized light motivates further work. Sensitivity and specificity will determine whether polarimetry contains sufficient information for definitive positive tumor margin detection, or whether using polarimetry as a guidance mechanism (for accurate but slow classification via mass spectrometry) is more appropriate.

S1-26. Optical Enantioseparation of Chiral Molecules Using Asymmetric Plasmonic Nanoapertures

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Using electromagnetic modeling and analytical methods, we study the optimal conditions of an enantioselective optical process (EOP) through the interaction potential between enantiomers and localized chiral near-fields created by asymmetric Plasmonic Crescent Monn (PCM) nanoapertures when it is illuminated with circularly polarized light. We introduce a chiral dissymmetry factor which measures the degree of chiral discrimination of the EOP and we found that it depends mainly on the differential field intensification, near-field optical chirality and the

handedness of the enantiomers. Our results prove that a sub-10-nm non-magnetic enantiomer pair of chiral spherical molecules with normalized chirality parameter up to ±0.003 can be passively separated under dual-symmetric conditions. The method and the proposed nanostructure may enable all-optical enantioseparation of single-chiral macromolecules such as proteins and carbohydrates.

S1-27. Study of the nonlinear optical properties and oxidative stability of comestible oils using the Z-scan technique

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Most oils of vegetable origin are characterized by having organic compounds in their chemical structure, they have large dielectric properties due to their chemical bonds, which gives them a high non-linear response, for these reason, this compounds are subject to rigorous research in the area of photonics and non-linear optics. have been implemented diferent studies for the evaluation and monitoring of the parameters that are implicated with the quality and utility of oils. Many of studies is realized usin wet methods (using a solvent), fuorescence analysis or spectroscopic methods; although many of these methods are invasive. Therefore the Z-scan technique has great feasibility to apply to the study of these oils, because it is a non-invasive technique and does not require other compounds or solvents, besides having high experimental simplicity and great sensitivity. In this study the nonlinear optical response of 4 vegetable oils was analyzed: extra virgin olive oil (EVOO), extra virgin sesame oil (EVSO), extra rened linseed oil (ERLO) and virgin avocado oil (VAVO), using the Z-scan technique; where the third order electric susceptibility value, refractive index, and characteristic non-linear absorption coeffcient were determined, parameters that were found by measuring the transmittance accordding the position of the sample, using a Nd: YAG laser operating in CW mode with a wavelength of 532nm. In addition, The extra virgin olive oil was selected for additional study, where samples of olive oil was submitted to thermal stress to evaluate oxidative stability, again using the Z-scan technique and thus, examine the relationship between nonlinear optical properties with chemical changes resulting from heating.

S1-28. Comparison of the quality of images with different supercontinuum pulse characterization techniques in nonlinear optical microscopy

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The in-depth study of the basic units of life requires the ability to probe deeper into cells and tissues in order to fully comprehend their functions. In recent years, multiple linear optical imaging techniques (Confocal, STED, Single Molecule Localized Microscopy etc.) have been developed in a number of different laboratories, all aimed at probing deeper into biological tissue and enhancing image quality and resolution. Many of these advantages obtained with these advanced linear imaging modalities are inherent in multiphoton imaging techniques (SHG, two-photon fluorescence, THG, CARS). These nonlinear optical microscopy (NLOM) techniques have the added advantage of intrinsic 3D imaging with submicron spatial resolution, decreased photodamage to tissue, increased depth of penetration as well as the ability to perform label-free imaging. These techniques depend nonlinearly on the spectral, temporal and spatial properties of the excitation source. Many NLOM techniques focus on image enhancement through the manipulation of the spatial properties of the source. In this presentation, we look at how the manipulation of the temporal properties of the excitation source contributes to quality of images. The processes in accessing the temporal features of the excitation source through supercontinuum generation from a highly nonlinear all normal dispersion photonic crystal fiber (ANDi-PCF) will be introduced. We compare two temporal pulse characterization techniques: Multiphoton Intrapulse Interference Phase Scan [1] and a novel pulse characterization technique based on Time domain ptychography [2,3] and how these contribute to the quality of the images recorded. Second harmonic generation in the guard cells of plants using these techniques will be discussed.

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S1-29. Improvement of microshear bond strength after femtosecond laser ablation of smear layer on sound and eroded dentin

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In restorative dentistry, the adhesion of resins to the dentin is still problematic in some cases. The conditions of the tissue (sound or eroded), and the commonly-used acid etching, induce tissue alterations that compromise the adhesion. This study uses femtosecond (fs) lasers pulses to promote controlled smear layer removal, improving the microshear bond strength (μSBS) of an adhesive system in sound and demineralized dentin. Twenty (20) human dentin samples were assigned to sound (SD) and demineralized dentin (ED) groups. All samples, except the control groups, were irradiated with two different fluences (11 and 18 J/cm2). A self-etch adhesive system was applied to bond a resin composite to measure the μSBS. Each procedure was repeated 10 times for statistical considerations (n=10) using Mann Whitney ($p < 0.05$) and Kruskal Wallis ($p < 0.01$) tests. Fracture analysis was performed using an optical microscope. The irradiated surface was assessed via OCT images, also used to measure the ablated volume. Scanning Electron Microscopy and Atomic Force Microscopy were also applied. The optimum bond strength was recorded for the SD group etched with 11 J/cm2, and the minimum bond strength was recorded for ED. Statistical analysis confirmed significant differences between the experimental groups. The imaging techniques showed the opening of dentinal tubules and a higher laser-induced roughness, which may be responsible for enhancing the bond strength. In conclusion, the plasma induced by femtosecond laser pulses successfully removed the smear layer of sound and eroded dentin, producing morphological alterations that can improve the dentin-resin adhesion.

S1-30. Desenvolvimento de nanoestruturas em superfície metálica (prata) com laser pulsado femtossegundo para aumento de fluorescência

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Laser Induced Periodic Surface Structures (LIPSS) are structures formed from the interaction of a high-energy laser with a sample. In the process of creation of these structures, the surface electrons absorbs the energy, the metallic structure melts, and this process takes a few nanoseconds, and affects the surface in the nanometer scale. With the reestablishment of the thermal equilibrium, the metal solidifies and self-organize its structure and nanostructures are formed. This occurs due to the absorption of energy and deformation of the metal crystalline structure with the cumulative effect of absorption, heating, melting, restructuring and fast freezing for each pulse over the surface. The aim of this project is to study the nanostructures formation process and the. We want with this to amplify signals of fluorescence and make possible the use of metal nanostructures for Metal Enhanced Fluorescence. We used a Titanium-sapphire femtosecond laser from Coherent/Libra with 1KHz of repetition rate, average power of 1.0watt and wavelength between 780-820nm and polished bulk silver surfaces to be marked. Some SEM images shows the best nanostructures formed using 5mm/s of laser scanning speed and line separation of 0,001mm, which grants around 36-pulse overlapping. Using Protoporphyrin-IX to test the fluorescence amplification, we obtained around 7 times the fluorescence signal compared with a surface without the nanostructures. The next step is to test a greater range of parameters variation and try the deposition of PpIX inside a polymer over the silver surface for better interaction between the nanostructures and the fluorophore.

S1-31. Nonlinear optical microscopy and bioimaging

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In last decades, nonlinear optics microscopy has become an attractive tool in biomedical research. To overcome some limitations, induced by fluorescence microscopy by exploiting nonlinear optical effects novel microscopy techniques have been developed, which afford high chemical selectivity of unlabeled living cells, and in addition, implementation real time three-dimensional imaging with high spatial resolution and sensitivity. In this work, we focus the attention on Stimulated Raman Scattering (SRS) technique. This technique is sensitive to the same molecular vibrations probed in spontaneous Raman spectroscopy, but unlike linear Raman spectroscopy, it exhibits a nonlinear dependence on the incoming light fields and produce coherent radiation.We describe the design and the implementation of a microscope based on femtosecond Stimulated Raman scattering (f-SRS), which is able to cover all the regions of Raman spectra: the fingerprint region (400 cm-1 – 1600 cm-1), the silent region and the C-H region (>2700 cm-1). The experimental imaging setup is equipped with three femtosecond laser sources: a Ti:Sapphire (Ti:Sa), a femtosecond synchronized optical parametric oscillator (OPO) and a second Harmonic Generator (SHG). In order to cover all the regions of Raman spectra, they can be used in two different combinations. The first one, using Ti:Sa and OPO+SHG, we can cover in SRL modality the fingerprint region and the silent region. The second one, using Ti:Sa and OPO, we can cover the C-H region in SRG modality. The system, not commercially available, is the result of the integration of a femtosecond stimulated Raman spectroscopy set up with C2 confocal Nikon microscope, which is made up by an inverted Nikon Ti-eclipse microscope and a scan head.

S1-32. Investigating the effect of photodynamic therapy in bone formation using polarimetric second-harmonic genration microscopy

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Photodynamic therapy (PDT) is a form of phototherapy based on activation of a photosensitizer by light. This photochemical reaction generates cytotoxic species inducing apoptosis and/or necrosis of targeted cells and tissues. It has been previously shown that the there is an increase in strength, stiffness, and architecture of bone in metastatically-involved vertebrae which received PDT treatment. In addition, it was shown that applying PDT treatment on comminuted rat tibia fractures, increases new bone formation. Hence, it can be hypothesized that PDT treatment can also enhance the healing process and/or prevent the development of non-union in femoral fractures.Collagen type I fibrils are one of the major components of bone and its structure and the 3D organization play key roles in the bone strength. Since collagen has a noncentrosymmetric structure, it can generate second-harmonic generation (SHG) signal. Therefore, SHG microscopy can be used to investigate the structural properties of collagen. In this study, fracture model in a femur of 30 adult rats were studied using a polarization-resolved SHG microscopy technique. Three groups of rats were formed and the rats were randomly allocated to control group, and PDT applied groups either 1 day or 7 days after fracture generation (1d-PDT and 7d-PDT, respectively) each with 2 survival time points (7 or 15 weeks).A difference between the old and the new bone in the control sample was observed indicating that the newly formed bone in the new bone is not as well-organized. On the other hand, no significant difference was observed between the old and new bone in the 7d-PDT group indicating that the remodeling in the 7d-PDT group was further ahead compared to the control and 1d-PDT group. All the significant differences disappear in the 15-week survival group because the bone in all cases had more time to remodel and to come closer to the original collagen orientation state.

S1-33. Investigation on physical,chemical, microscopic and molecular biological characteristics of fungal biodeterioration in diferent cultural heritage materials

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Conserving the cultural heritage (CH) and works of art is a general concern, because they are constantly exposed to biodegradation due to the presence of micro-organisms such as bacteria, archaea, fungi, algae, as well as lichens and insect pests (biodeteriorating agents). The analytical study of materials in CH objects has to be noninvasive and nondestructive in order to preserve their integrity. The disinfection or decontamination technique to be applied is Irradiation 60Co by developing a dosimetry protocol. For characterization CH object selected, imaging and spectroscopy-based on Ion Beam Analyses (PIXE/PIGE/RBS) and FT-IR-ARM analytical methods and protocols are developed and standardized at LAMFI at USP. Furthermore, the study of CH, archeological and works of art products using the protocols developed may provide an identification and analysis of the material employed, era, geographic localization as well as the techniques used by the artist. Therefore, proper conservation and preservation methods are necessary for ensuring a long service life of CH and work of art products, including ethnographic and historic documents, paintings, archeological finds kept in a museum, church, and repositories stored in the archives of the Museums at USP in Brazil . The objectives are to classify these samples among those deteriorated by the presence of microorganisms poor state of conservation , to develop protocols of analytical techniques predominantly non-invasive and non-destructive imaging and spectroscopy, PIXE/PIGE/RBS, FT-IR/ATR for application on these pieces of art without being damaged, in order to preserve their integrity.

S1-34. Optical characterization of synthetic metabolic fluorophores: NADH and FAD

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Cell metabolism is the set of biochemical processes that occurs in an organism to energy production, cellular development and maintain cell vital functions. Monitoring cellular metabolism can be used to distinguish between healthy and abnormal cells. It can be done by observing some cellular organelles that have an important role in cellular metabolism. In this context, mitochondrial activity can be monitored by native fluorescent electron carriers called NADH and FAD. The optical redox ratio can be calculated between these two coenzymes can be used to estimate cell metabolic process. Therefore, optical characterization of these and other biomolecules is essential to the monitoring cellular metabolism. In this work NADH and FAD solutions were prepared in different concentrations and, interactions between fluorophores and medium were evaluated by fluorescence. Measurements of stationary state fluorescence and fluorescence lifetime were performed to characterize these biomolecules in solution. For steady-state fluorescence comparison between the two fluorophores, an excitation-emission matrix (Ex. 250-500 nm, Em. 300-650 nm) was set up for each one of the solutions. Fluorescence lifetime was measured by TCSPC, using a 20 MHz pulsed laser emitting at 378 nm and processed in MATLAB. Average fluorescence lifetimes were calculated also for comparison. Results indicates average lifetime of NADH decreased from 2.10 ns to 1.10 ns when comparing 20uM NADH solution with 20 uM NADH and 20 uM FAD. While excitation-emission matrix showed a possible energy transfer between the fluorophores when concentrations achieved 20uM NADH and 0.2uM FAD. Finally, the next step is to prepare solutions with different biochemical properties and to evaluate its optical differences.

S1-35. Study of coupling agent influence in the light propagation

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When light strikes a biological tissue, it can undergo different types of interaction: reflection, refraction, scattering, transmission, and absorption. The way light interacts with a tissue depends on the optical and structural characteristics. In phototherapy and photodiagnostic procedures, dosimetry is usually done based on empirical data and experiments published in scientific articles, little is done to customize dosimetry in clinical practice, although it is known that the color, hydration, and roughness of the tissue surface may influence the propagation

of light. In this work, computational simulations of light interaction were carried out with a homogeneous turbid medium whose optical properties are equivalent to those of the skin dermis. The simulations were done using the Monte Carlo method and, for this, the implementation developed by FANG called MCXLAB. The aim of the present study is to evaluate how the propagation of light within the turbid medium depends on the coupling of light on its surface. For this, in the simulations, the existence of a layer of material above the turbid medium, with different optical characteristics was considered. The results show that the addition of a transparent material above the turbid medium changes the shape of the light propagation, and this shape depends on the refractive index and the thickness of the material added.

S1-36. Strategies for the Immobilization of Photosensitizers onto Solid Supports

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Nowadays, photodynamic therapy (PDT) is considered a promising alternative for killing cancer cells and pathogenic micro-organisms, due to its high specificity and low secondary effects.[1] One of the ways to maximize PDT activity is based on the development of ideal photosensitizers (PS) and light sources, aiming to modulate their photophysical and photochemical properties. Among the many photosensitizers studied so far, tetrapyrrolic macrocycles have received great attention due to their easy synthetic modulation, high activity and selectivity for PDT applications.[2] More recently, the use of immobilized photosensitizers onto solid supports began to receive increased interest for in vivo PDT application due its high loading capacity, protection against degradation, long circulation time and selective targeting.[3] In addition, the immobilization of photosensitizers onto solid supports is also a challenging way to clean clinical surfaces/materials contaminated with pathogenic micro-organisms.[4] Herein, we present our recent achievements on the development of efficient synthetic methodologies for functionalized porphyrins aiming at their covalent immobilization onto functionalized magnetic nanoparticles and carbon materials, namely nanodiamonds, whose studies concerning the assessment of their PDI effects are currently underway. The new immobilized photosensitizers were characterized by X-ray photoelectron spectroscopy (XPS), ultraviolet–visible spectroscopy (UV-Vis), Fourier-transform infrared spectroscopy (FTIR) and fluorescence emission spectroscopy.

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S1-37. Synthesis and in vitro biological activity of hypericin glucamine derivative for application in Photodynamic Therapy

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Hypericin currently considered as a third generation photosensitizer, due to its chemical characteristics, changes from its monomeric state to the aggregate state when left in biological medium, thus reducing its photodynamic effects. In this case, molecular alterations to decrease the state of aggregation are necessary for this compound to be applied in medical therapy. The aims of this study was synthesis and characterization of hypericin and hypericin derivative (hyp-Glu), to compare the aggregates formation for both structures and evaluate in vitro biological activity in MCF-7, MDA-MB-231 and HaCat cell line.The compounds were characterized by 1H NMR and infrared spectroscopy and mass spectrometry. Photophysical and photochemical properties were analyzed by absorption, excitation and emission spectra. The synthetic, chemical and photochemical steps indicated that hypericin derivative have higher relative efficiency in the production of singlet oxygen than hypericin and also have lower rate of aggregation in biological medium.For in vitro studies, MCF-7 cells was used to evaluate the toxicity and phototoxicity of hypericin and hyp-Glu, intracellular localization, mutagenic and genotoxic capacity,

clonogenic capacity and path of cell death identification. In order to compare the toxicity results of hypericin in other cell types, the cellular toxicity study was performed for MDA-MB-231 and HaCat cells.In in vitro assays it was verified that the effectiveness of the photodynamic activity was higher for the hyp-Glu, when compared to the hypericin, demonstrating that the molecular modifications in the hypericin macrocycle caused changes in the interaction between photosensitizers and the cell line studied.

S1-38. Monitoring of Radachlorin photobleaching kinetics in different cell lines in vitro

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Photodynamic therapy is a promising tool for treatment of various medical conditions. In the last several years considerable efforts have been made aimed to development of new photosensitizers and treatment protocols. Many studies were devoted to identification of cell death mechanisms and selective targeting of cancer cells by specific photosensitizers. Our work concerns monitoring of intracellular photosensitizer fluorescence and its temporal parameters, photobleaching kinetics in particular. The study was performed on HeLa and A549 cell lines using Radachlorin photosensitiser, which mainly contains chlorin e6 with minor additives of purpurin 5 and chlorin p6. Accumulation of photosensitizer by living cells was achieved by dissolving Radachlorin in DMEM culture media and further cells incubation during 24 hours. A set of time-resolved intracellular fluorescence images were acquired by means of highly sensitive CMOS sensor, and processed under assumption of single-exponential bleaching model. Numerical processing of the obtained data allows to reconstruct spatially resolved image of the bleaching rate in live cells. According to our studies this parameter may vary among different cell lines, and in various intracellular components. It can be explained by different amount of generated intracellular singlet oxygen which can cause significant variations in photobleaching rate of the photosensitizer molecules. Moreover for different cell types we have observed significant variations in Radachlorin accumulation level and cells response to the same dose and mode of photodynamic treatment. The results obtained correlate well with our recent studies of cell death dynamics obtained using digital holographic microscopy.

S1-39. Phototoxic Effect of Aluminum Tetra-sulfonic Phythalocyanine on Chemo-Resistant Breast Cancer Cells.

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Breast cancer represents the most lethal type of cancer among women. Conventional chemotherapeutic treatments have failed due to enhanced self-renewal capacity and the over expression of ATP binding-cassette (ABC) transporters by resistant populations of the cells. Hence it is significant to identify and evaluate a novel therapeutic approach. The use of a photosensitizer (PS) and light to produce death-inducing levels of reactive oxygen species in Photodynamic therapy (PDT) has the potential to meet the unmet common medical needs. In this study, we aimed to develop a resistant MCF-7 breast cancer cells (MCF-7/DOX) and study the novel photodynamic treatment efficacy. The MCF-7/DOX cells was developed by continuous exposure to increasing concentrations of Doxorubicin (DOX) treatment and characterized for P-glycoprotein (P-gp) expression using flow cytometry, fluorescence microscopy and enzyme immunoassay. Subsequently, MCF-7/DOX cells were treated with Aluminum tetra-sulfonic phthalocyanine PS for 4 h and irradiated at 5, 10 and 20 J/cm2. Thereafter, the cells were incubated 24 h post-irradiation before biochemical assays to check the cellular viability, cytotoxicity and proliferation. Our results revealed that irradiation of PS treated cells showed significant photodynamic therapeutic efficacy on MCF-7/DOX cells with low mitochondrial enzymatic activity and cell viability. In addition, there was increased lactate dehydrogenase enzyme leakage followed by PDT experiments. These results indicate that PDT might be an effective treatment modality for multidrug resistant tumors.

S1-40. Photoinactivation effect of chlorophyllin sodium-copper against Staphylococcus aureus

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In view of the emerging need for alternatives to control the growth of bacteria involved in infectious processes, it is necessary to propose new alternatives, because if nothing is done, we will have a devastating problem in 2050 [1]. The present study evaluated the potential application of chlorophyllin sodium copper salt (CuChlNa) in Staphylococcus aureus (S. aureus) ATCC 25923 and S. aureus MRSA (Genbank accession number Mh087437). The experiments were performed using S. aureus strains (MRSA methicillin-resistant and MSSA methicillin-sensitive) in which five photosensitizer (PS) concentrations (0.0, 1.0, 2.5, 5.0, 10.0 and 20.0 μM). After a PS incubation, the samples were divided in two groups, one kept in the dark and another was submitted to illumination. Then, the bacterial inactivation was determined 18 h after the incubation at 37 °C by counting the colony-forming units (CFU). The results obtained showed that CuChlNa can be applied as a effective PS for Photoinactivation in S. aureus ATCC and S. aureus MRSA. These results demonstrate that bacterial growth was completely inhibited at concentrations over 5 μM of PS for S. aureus (MSSA) and over 10 μM of PS for S. aureus (MRSA) when irradiated by red light using a dose of 30 J/cm2.These study demonstrated that CuChlNa have a great pontencial for application in PDI, preseting a high molar absorption coefficient in the 600-800 nm rang and as being non-toxic in the dark.

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S1-41. Supramolecular G-Quadruplex Hydrogel as a Photosensitizer Carrier

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Self-assembly of guanosine is an efficient way to form biomaterials, such as supramolecular hydrogels with promising applications. Guanosine form cyclic planar units, called G-quartets, through hydrogen bonds (Hoogsteen). In presence of monovalent cation, G-quartets stack through pi-pi interactions resulting in columns: the G-quadruplex. Depending on the conditions, G-quadruplex is able to make a 3D-network with high capacity to entrap water (Carducci et al., 2018). Our study has focused in getting stable hydrogels by the self-association of two precursors: Guanosine (G) and Guanosine 5'-monophosphate (GMP), varying the proportion between them. The ability of these gels to trap and release photosensitizer as Methylene Blue (MB) was analyzed. Three different proportion between G and GMP (1:6; 1:2 and 1:1) were used for the following experiments. Small Angle X-Ray Scattering (SAXS) was performed to infer about the structure. Higher structural factor peak was observed to lower proportion of GMP, indicating that 1:1 gel presents the strongest lateral interaction between the fibers. The MB solution was deposited on the top of the gel to check its diffusion into G-quadruplex network. The MB was able to penetrate homogeneously into hydrogel without mechanical stress. The velocity was proportional to GMP (negative charge). The total penetration was achieved with 270 min to 1:6; 390 min to 1:2 and 600 min to 1:1. Different patterns of fibers were seen on the Atomic Force Microscopy (AFM) images for different proportion G:GMP. In presence of MB, no significant changes were detected on the aggregation type seen by SAXS and AFM. A pH dependence releasement of MB from hydrogel was also verified. Guanosine hydrogel is a biocompatible and biodegradable material to be explored as a bioactive carrier and further can be used to encapsulate photosensitizer to be applied in Photodynamic Therapy.

S1-42. Host-guest complex of β-Ciclodextrin and Hypericin enhaces photoxicity in HeLa cells

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Photodynamic Therapy (PDT) is a therapeutic modality based on the administration of a nontoxic compound known as photosensitizer (PS) followed by visible light irradiation inducing an oxygen-dependent cell death. Hypericin (HY) is a PS largely used in PDT of cancer. However, HY is poorly soluble in aqueous medium, which restrains its bioavailability and decreases the PDT efficiency. β-Cyclodextrin is a type of cyclic oligosaccharide

that has seven glycopyranose units, forming a hydrophobic internal cavity and hydrophilic external surface which allows host-guest complexes formation with other molecules, essentially hydrophobics. In this study host-guest complex between HY and β-CD, named as β-CDHY, was synthesized by the co-solvent method with sonication. The evidences of complex formation were given by infrared spectroscopy and NMR-1H. A complexation efficiency of 42% was obtained and HY became soluble after complexation. No changes in photostability and fluorescence quantum yield were observed. Cell uptake and phototoxicity of the complex were evaluated in HeLa cells after 2 h of incubation and it was noticed a twofold increase in cellular uptake and an improvement of 40% in phototoxicity after irradiation with a 590 ± 10 nm LED with a light dose of 10 J cm-2 and 8.4 mW cm-2. The increase in phototoxicity can be justified by the increase of water solubility of HY after the host-guest complex formation, which consequently enhances its diffusion in physiologic medium, besides, improves the cell uptake. These advantages suggest that β-CDHY has potential for use in PDT.
Key words: Hypericin; β-Cyclodextrin; Photodynamic Therapy

S1-43. Human Physiological Parameter Monitoring Using Non Contact Technique

Preeti Jagadev (PhD Scholar at National Institute of Technology Goa) and Dr Lalat Indu Giri (Assistant Professor at National Institute of Technology Goa)

The Infant Respiratory Distress Syndrome (IRDS) is one of the leading causes of death in premature neonates. It's very alarming that one out of ten babies is born premature, with the number increasing in recent years. The ever increasing number of critical cases and the limited availability of skilled medical attendants, advocates in favour of a reliable neonatal monitoring system. The existing contact monitoring processes often pose problems for premature neonates having fragile skin, making them prone to infections. These problems can be overcome by making use of a peculiar branch of bio photonics, which provides measurement, visualization and quantitative analysis of the infrared radiation emitted from the human beings. Infrared thermography which is a non-destructive, non-invasive and a non-contact approach is a viable alternative which deals with these concerns. The Focal Plane Array based IR cameras detect the variation in temperature across the nostrils of neonates during breathing, in a non invasive manner. The obtained breathing signal has a low SNR , which is improved by subjecting it to well established filtering techniques. Various statistical parameters such as rise time, fall time, wavelength and periodicity of the filtered breathing waveform are then extracted, along with the emissivity of human skin and mean temperature of the nostril. A peak detection algorithm is designed, to determine the number of peaks in the filtered breathing waveform and further obtain the respiration rate. The robustness of the algorithm is tested under various conditions such as constant respiration, rapidly increasing respiration, holding breath, shallow breathing, head motion, close camera focus and distant camera focus. The performance of the algorithm is determined by computing the precision and sensitivity value. The respiration rate obtained is validated with complementary established medical techniques, to test the accuracy of the proposed system.

S1-44. General formula to eliminate spherical aberration produced by an arbitrary number of lenses in a Microscope

Rafael G Gonzalez-Acuña and Julio C. Guitierrez-Vega (Tecnologico de Monterrey)

We present a general analytic and close-form formula to determine the shape of a surface that corrects the spherical aberration generated by an arbitrary number of preview refractive surfaces. The preview surfaces must be such as the rays inside the optical system do no cross each other. The close-form formula presented here in fact is the general formula of the microscopes design, if we take the distance of the object to the first surface as finite. Therefore with this new close-form expression countless of microscopes can be designed with the characteristic that all of them are free of spherical aberration and have a high quality image.

S1-45. Techniques of optical trapping through Generalized Phase Contrast

Pedro Faleiros Silva, Sérgio Ricardo Muniz, Thalyta Martins, Charlie Oncebay Segura (Sao Carlos Institute of Physics - University of Sao Paulo, Brazil)

The motivation of this project is to develop experimental techniques of optical trapping and optical control applicable to atomic physics systems and nanostructures control as a platform for the study of important contemporary problems of modern physics and technology - which is in the context of the generation of optical potential arbitrary systems to simulate out-of-equilibrium systems, coherent control of qubits, and quantum simulation. This idea can also be applicable in the context of biological structures control and in optogenetics through neurological optical design. In particular, in this project will be studied the spatial modulation of a light beam by the Generalized Phase Contrast (GPC) technique, to produce, with very high precision, different optical potentials. The literature of this type of technique for optical trapping is recent and this project will contribute to its development and its diverse application through the systems already present in the laboratory of quantum technologies of the Optics Group of the Physics Institute of São Carlos.

S1-46. Noninvasive holographic monitoring of various cells response to photodynamic treatment: comparative analysis

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The significant recent advancements in photodynamic therapy and a wide range of novel photosensitizers being developed stimulate research and analysis of processes associated with photodynamic treatment. The high photosensitivity of photosensitized cells poses significant difficulties to investigations of their death dynamics by means of fluorescence microscopy. Our work concerns application of digital holographic microscopy and tomography for monitoring of optical and morphological parameters of cells after photodynamic treatment at various treatment modes. The technique is based on monitoring of phase distributions in object wave, it is completely noninvasive and does not require cells staining or exposure to intense illumination. At the same time it allows to obtain quantitative data on several optical and morphological cellular parameters. In our research we monitor the dynamics of average phase shift variation in cells, and we have demonstrated that this value can be used for identification of the cell death mechanism and estimation of its typical duration. For instance, apoptosis can be observed at low doses of photodynamic treatment, and higher doses result in cells death through necrosis. The analysis of cell death dynamics has shown that necrosis rate significantly depends on irradiation power density and cells type. Different cell lines demonstrated substantially different response to the same PDT procedures. For example A549 cells show significantly higher resistivity to generated intracellular ROS as compared to HeLa cells. We have also performed research on cell specimens prepared from neoplastic material taken from patients and transferred into a culture. It was found that cells of the same type, taken from various patients may show significantly different response to photodynamic treatment with the same dose. Our further research is aimed at comparative evaluation of various photosensitizers efficacy in photodynamic treatment of various cell lines.

S1-47. Hyperspectral imaging of human skin aided by artificial neural networks

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The aim of our study is the development of a new approach to hyperspectral imaging for quantitative diagnostics of skin including 2D mapping of skin chromophore distribution, kinetics of blood oxygenation maps and evaluation of skin perfusion.Two-dimensional spatially resolved diffuse reflectance of human skin was acquired with a compact hand-held hyperspectral camera. A laboratory-build broadband illumination setup is based on a fiber-optic ring illuminator providing uniform distribution of light intensity. The system of crossed polarizers was used to reduce the effect of specular reflection from the skin surface.To solve the inverse problem of estimation of both

skin chromophore content and the values of blood oxygenation, an approach based on the neural network has been chosen. A training set of spectra for the neural network was modelled by the Monte Carlo method. A CUDA-based Monte-Carlo distributed computing platform was used for routine simulation of diffuse reflectance spectra. The differences in the spatial distribution of blood, melanin, oxygen saturation of blood, water content within the skin, as well as the detector parameters were taken into account.The developed hyperspectral imaging system was used to perform trial measurements and the occlusion tests with healthy volunteers. The system has shown the ability to sense the alterations of blood and melanin content as well as blood oxygen saturation. The implementation of the neural network-based processing allows for fast recovery of the considered skin parameters. The combination of a fast and compact hyperspectral camera with a broadband light illumination and neural network-based processing allows for multiparameter estimation of tissue properties. Potential applications of the proposed technique are of high social impact and include monitoring and diagnostics of diabetic ulcer formation, rheumatic complications, diagnoses of melanoma and other malignancies, quantitative skin screening and others.

SESSION 2 - 26 March, 2019 - 4PM

S2-01.Photophysical Properties of Ir(III) complexes and their biological application

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In recent years, the photophysics of d6 metal coordination compounds has been the subject of extensive studies due to its high stability in the presence of several ligands, allowing a variety of optical and electronic properties that can be conveniently applied in solar energy conversion systems in electric, as in dyes sensitized solar cells, or in systems of converting electric energy into light, such as organic light-emitting diodes and light-emitting electrochemical cells [1].Iridium (III) complexes specially have found a range of biological applications, including biological markers, chemical sensors and photodynamic actions. Compared with other transition metal complexes, its photophysical properties are favorable; the polypyridine complexes of Iridium (III) show tunable emission over a wide range of the visible spectrum and even part of the NIR region through the use of diferente coordination ligands[2] . In addition, iridium (III) complexes exhibit good photostability and large Stoke's shifts that can minimize luminescence self-quenching.Given the large potential, it is expected that the applicability scope of the Ir (III) complexes will be expanded. The knowledge accumulated in this work, on synthesis and photophysical processes, such as light absorption, excited state deactivation, sensitization and quenching of Ir (III) complexes will aid in the future development of biologically relevant photofunctional molecules to be developed by the LEMAF/ IFSC group .

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S2-02.Exosomes from breast tumor cells and their role on cell adhesion during metastasis process. In vitro studies

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Exosomes are bilayered membrane vesicles of 30-150 nm released by tumor and healthy cells into interstitial spaces and body fluids. They contribute to tumor spread, being delivered to both tumor microenvironment and pre-metastatic niche, carrying a set of molecules and genetic material that transfer crucial information to recipient cells. Integrins are adhesion receptors found in exosomes and believed to play important roles on exosome function since these vesicles interact with other cells or with the extracellular matrix. Here, we report the role of

exosomal avb3 integrin on adhesion and uptake of exosomes, by blocking them with the recombinant RGD-disintegrin DisBa-01. Western blotting of exosomes showed the presence of the validated target for DisBa-01, the avb3 integrin. Either exosome adhesion on fibronectin and collagen coatings as well as the uptake on MCF10A cells (healthy breast cells) were significantly reduced upon treatment with DisBa-01. When exosomes were used as coating in cell adhesion assays, a positive effect on cellular attachment was observed, which was decreased by DisBa-01 treatment. Moreover, in GFP-CD63 MDA-MB-231 cells co-cultured with MCF10A cells, it was clear that the amount of vesicles delivered to healthy cells was considerably decreased after integrin inhibition. Our findings demonstrated the participation of avb3 integrin on cell communication through exosomal pathway, bringing light to a new approach for integrins as targets on tumor development inhibition.

S2-03. In vivo effects of stereotactic body radiation therapy (SBRT) on the pancreatic cancer microenvironment

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Pancreatic cancer (PC) has one of the lowest 5-year survivals of any major cancer due to its aggressive and treatment-resistant phenotype. Desmoplastic stromal cells in the tumor microenvironment (TME) are believed to play a pivotal role in facilitating therapeutic desensitization of PC. This has intensified research efforts in identifying new strategies to target both tumor cell and stromal components of the TME for treatment. Stereotactic body radiation therapy (SBRT) is an emerging therapy for treating PC that delivers conformal radiation using image-guidance, minimizing toxicity and treatment length. While SBRT is more promising at providing local control, the rate of distant metastasis remains high. Thus, a better understanding of the radiobiological changes to the tumor stroma caused by SBRT may help uncover new strategies to improve the efficacy of SBRT for PC patients.In this study, we optimize an orthotopic mouse model of PC, a small animal x-ray micro-irradiator, and intravital fluorescence microscopy to evaluate the effects of SBRT on the pancreatic TME. First, an mNeptune-Luciferase-Panc02 cell line is inoculated into the pancreas of a mouse. A custom-made abdominal window chamber is then surgically implanted over the tumor site to visualize the tumor. SBRT can then be delivered using an optical bioluminescence-guided targeting system (X-RAD 225Cx, Precision X-Ray Inc). In vivo fluorescence and second-harmonic generation images can be taken through the window chamber before, during, and after therapy using a laser-scanning confocal microscope (LSM710, Zeiss). This pre-clinical experimental platform can be used to characterize the effect of SBRT on tumor vasculature and hypoxia, as well as desmoplastic stromal cells and fibrillar collagen in the tumor. Observing how these stromal elements respond to SBRT in vivo will improve our understanding of how pancreatic tumors develop treatment-resistance and subsequently invade the surrounding tissues.

S2-04. Plasmonic Metasensor for Wide Range of Refractive Index Sensing

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Plasmonic sensor is one of the highly efficient and effective optical sensing technique due to its fast response and extremely sensitive nature, enabling applications ranging from medical diagnostics, biochemical, environmental monitoring, and food safety to security [1, 2]. Considering the light controlling capability and miniaturized structure, metasurface based plasmonic sensors have shown great development and advantages recently. Recently, Liu et al. have reported an all-metal metasurface based plasmonic sensor which shows the maximum sensitivity of 400 nm/RIU in the sensing range of 1.312 to 1.352 [4]. Another interesting approach, using 4-packed silver nanodisks based all-metal plasmonic sensor has been reported by Zhengdong et al. [5], which shows the maximum sensitivity of 885 nm/RIU in the sensing range of 1 to 1.05. Although, to date several metasurface

based plasmonic sensors have been reported however, they are limited in the sensing range as a result extensive study is required to detect the lower analyte RI to higher analyte RI, simultaneously. In this work, we investigated a highly sensitive all-metal based plasmonic sensor which is fabricated following the electron beam lithography (EBL) process. Proposed sensor shows the maximum sensitivity of 910 nm/RIU with the average sensitivity of 630 nm/RIU over the sensing range of 1 to 1.60. The proposed sensor is able to detect the 10−4 scaled smallest RI changes of the samples. It also shows the highly linear sensing response of R2=0.9998. Due to promising results, proposed sensor will be a suitable candidate for the environmental monitoring, medical diagnostics including biological analyte, biochemical, organic chemical as well as high refractive index chemicals detection.

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S2-05. Quantifying c-di-gmp during infections on a single cell level

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The second messenger cyclic di-guanosine-monophosphate (c-di-GMP) allows bacteria to alternate between two important lifestyles, a sessile biofilm forming state and a motile virulent state. This switch is critical for the success of V. cholerae. A multitude of environmental and cellular signals effect the concentration of c-di-GMP. However, the changes in c-di-GMP and the corresponding phenotypes have not been characterized during infection. Here, we aim to quantify the intracellular level of c-di-GMP during infection. Currently, we are establishing fluorescence-based biosensors to measure c-di-GMP on a single cell level. We will calibrate these sensors with alternative, population based c-di-GMP quantification methods, testing strains with defined c-di-GMP levels. Once established, we will measure their response to isolated environmental signals and then proceed to use the biosensors during infections of a model host. This research will add to the knowledge of how external stimuli translate into rapid adaptation in pathogens. Furthermore, we will use our data to evaluate potential targets that interfere with the infection process by inhibiting V. cholerae adaptation to the host.

S2-06.Specific and Direct Amplified Fluorometric Detection of MicroRNA with MicroRNA:Argonaute-2 Cleavage (miRACle) Beacons.

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Molecular beacons (MB) are a class of fluorogenic sensors which consist of a fluorophore and quencher conjugated to an analyte-responsive linker. Generally, these linkers have fallen into two categories based on linker chemistry, yet recent investigations have favoured protease-targeting peptide linkers over hybridization-based nucleic acid linkers. This is largely due to the increased signal generated by multiple cleaved peptides vs. the signal generated by one target-hybridized oligonucleotide. However, the wealth of protease biomarkers is becoming exhausted, and there is a need to turn to new biomarkers while retaining the favourable activation profile of peptide-based beacons.To address these concerns, we turned to microRNAs, a class of over 5,000 oligonucleotide sequences, which exert their effect through the Argonaute (Ago) protein family. To access this massive class of biomarkers with a sensitive fluorogenic assay, we hypothesized that we could exploit the endonuclease activity of Ago-2, to create a new level of sensitivity and specificity to oligonucleotide-based linkers.We observed that our miRACle beacons underwent an Ago-2 mediated site-specific catalysis to generate 13x fluorescence increase vs. the classic hybridization mechanism. Moreover, using an enzymatic activation mechanism gave us additional functionality beyond hybridization beacons: a single mismatch completely abrogated beacon activation, likely due to the mismatch resulting in a bulge, impairing enzyme function. We were also able to easily adapt our system to use nuclease resistant non-RNA bases, potentially allowing miRACle beacons to be used in live cells. Lastly, to demonstrate the future potential of this easily adapted system, we designed a miRACle beacon targeting liver-specific miRNA miR-122, and demonstrated tissue- and site-specific

sensor cleavage potentially allowing tissue-specific imaging with the plethora of tissue-specific miRNAs discovered.

S2-07. Early and Non Invasive Detection of Breast Cancer on Layered Phantom

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Breast cancer is one of the most leading causes of death of women all over the world. Survival rate can be increased even up to 97 %, if it is diagnosed in the early stage and proper treatment is taken. It calls the attention of researchers to investigate reliable and efficient devices and methods to early detection of breast cancer. From last few decades, some standard diagnostic tools for e.g. mammography, ultrasound, and biopsy-based methods are being used for early detection of malignant tumors, but these methods are expensive, require trained people to handle, provide low sensitivity and give some hazardous radiations to patents. An antenna based device is demonstrated as a biosensor to detect tumors of breast cancer in very early stage and to categorize their classifications as a malignant or benign tumor. Finite element based CST suite module has been used for simulation purpose for different sizes, positions and types of tumors in a rectangular assumed phantom of breast. In order to validate the simulated result, experimental test of the device has been performed using Voltage Network Analyzer [VNA]. Breast phantom has been prepared by easily available materials like glass, petroleum jelly, mixture of water and wheat flour. The device is smart enough to detect the tumor of less than 1 mm in radius and located anywhere in 6 cm depth and 5 cm× 5 cm area of breast fat phantom. The proposed device has many advantages like it is noninvasive, easy to use, low cost, safe, comfort (as not the requirement of compression of the breast), non- ionizing.

S2-08. Creation of sensitive biological element for direct label-free biosensors by machine learning assisted patome primer database

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Direct recognition sensors, in which the biological interaction is directly measured in real time, typically use non-catalytic ligands such as cell receptors or antibodies. Such detectors typically measure directly physical changes (e.g., changes in optical, mechanical, or electrical properties) induced by the biological interaction and do not require additional labeled molecules (i.e., are label-free) for detection. Machine learning comprises models that learn from existing (training) data. A novel database of primers for PCR detection was built from preprocessed data originated from publicly available patented biological sequences (<50 nucleotides) at databases such as World Intellectual Property Organization (WIPO) and Cambia Lens with Python and framework Apache Spark/Apache Zepellin.

S2-09. Design and validation of a DNA biosensor for the quantification of proteins in blood

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Biosensors based on DNA allows building portable and disposable devices for protein quantification useful in point of care diagnosis. Conventional methods for quantifying proteins exhibit substantial disadvantages related to costs and difficulty of the technique when used in settings where fast and cost-effective assays are needed. In this work, we report the successful application of a simple, rapid, easy to use and labeled-free biosensor based on DNA aptamers. The protein detection strategy is based on the use of the selective fluorescence of the NMM IX dye with some oligonucleotides. This aptasensor uses a computational-aided designed thrombin recognition probe, which consists of two modules, a detection aptamer, and a transduction sequence that induces the specific fluorescence of NMM IX. In the presence of a specific protein, the membrane acquires a fluorescent spot visible to the naked eye. The fluorescent response is directly proportional to protein concentration and can be easily quantified colorimetrically using a low-cost microscopy system. The recognition probe design might be adaptable

to other relevant biological analytes by changing the sequence of the aptamer. This proof of principle represents a contribution to the development of useful, cheap, reliable, and simple protein quantification assays for point of care diagnosis.

S2-10. Organic Distributed Feedback Lasers Functionalized for Sensing Applications

Pedro Ramon Oiticica, Osvaldo Novais de Oliveira Jr.; São Carlos Institute of Physics, University of São Paulo.

Organic semiconductor lasers can be used for sensing applications[1][2], where lasing characteristics such as threshold, wavelength and losses are employed as the sensing mechanism. The low-cost easy fabrication and the advances in organic semiconductor laser materials with low lasing threshold makes them good candidates for lab-on-a-chip (LOC) devices. In our research proposal, we aim to develop a sensing systems based on recent advances in organic Distributed Feedback Laser (DFB laser) that opened possibilities for biocompatible sensor devices[3]. We shall focus on biosensors based on evanescent optical wave interactions of biomarkers on the surface of an organic DFB laser gain material. Specific biomarker detection can be achieved by functionalizing the surface of the gain material with probe molecules that specifically bind to the analyte. The sensing process is provided by the change of the surface refractive index upon the analyte binding. Most evanescent optical wave sensors are based on passive resonant structures such as dielectric micro-resonators, photonic crystals or surface plasmon resonators. Evanescent wave optical detection of the surface functionalized organic DFB laser is a label-free detection technique and promising for LOC devices.

[1]G. Mcconnell et al., “Organic Semiconductor Laser Platform for the Detection of DNA by AgNP Plasmonic Enhancement,” 2018.
[2]A. M. Haughey, G. McConnell, B. Guilhabert, G. A. Burley, M. D. Dawson, and N. Laurand, “Organic semiconductor laser biosensor: Design and performance discussion,” IEEE J. Sel. Top. Quantum Electron., vol. 22, no. 1, 2016.
[3]M. Karl et al., “Flexible and ultra-lightweight polymer membrane lasers,” Nat. Commun., vol. 9, no. 1, 2018.

S2-11. Fluorescence-based biosensor for the detection of microorganisms

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The rapid and precise identification of microorganisms constitutes an essential and extremely important stage in the diagnosis of bacteria and fungi such as Escherichia coli and Candida albicans, respectively. Therefore, the development of a method that is fast, efficient and inexpensive is necessary to detect microorganisms in environment, food and biological samples. In this sense, the objective of this work was to assemble a biosensor based on fluorescence technique for the detection of microorganisms. The biosensor basically consists of a FDS100 photodiode, LED UV emitting 1.6 mW at 280 nm and a bandpass color filter 314-445 nm. The system is controlled by an Arduino Uno R3 microcontroller coupled with the Android application. The Android application was developed to setup of the parameters such as delay time, power of laser, photodiode gain and to save the data achieved. The antimicrobial peptide (AMP) Polycerradin was used as bioreceptor in the biosensor assembling. This work presents great potential in the identification of pathogenic diseases due to portability and efficiency of the system if compared to other biosensors available. In addition to E. coli and C. albicans other bacteria species have been detected such as Staphylococcus aureus and Shigella sonnei due to the emission spectra of tryptophan of each bacteria.

S2-12. Kinetic Studies on the peroxyoxalate reaction using green oxalic esters.

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The peroxyoxalate system is formed by the reaction of oxalic esters and hydrogen peroxide in the presence of a base and an activator, and is one of the few high-efficiency chemiluminescence systems, reaching up to 50% of quantum yield, only comparable to enzymatic bioluminescence reactions. Over the past 25 years, this system has

gained importance associated with its applicability on analytical chemistry studies and more recently on the bioimaging area. This is due to its efficacy detecting hydrogen peroxide which plays a central role in causing several life-threatening human diseases, like cancer. Also, in typical chemiluminescence demonstrations and studies, the commercially available oxalate diesters, bis(2,4,6-trichlorophenyl)oxalate (TCPO) or bis(2,4-nitrophenyl)oxalate (DNPO), are used. These compounds are dissolved in volatile, nonbiodegradable, and toxic organic solvents. The products of the chemiluminescent reaction are carbon dioxide and either 2,4,6-trichlorophenol or 2,4-dinitrophenol, both of which are aquatic and terrestrial toxins. To avoid these toxic pollution sources, new chemiluminescent reactions have been designed employing not only oxalates synthesized from naturally occurring molecules but also nontoxic and biodegradable solvents (triacetin and ethyl acetate). These environmentally benign and commercially available analogs are derived from vanillin (DVO) and methyl salicylate (DMO). The use of all these four compounds(DVO, DMO, ethyl acetate, triacetin) might open up future applications of the peroxyoxalate reaction in bioimaging.

S2-13. Toxicity Assessment and Synchrotron X-ray Fluorescence Imaging to Localize Biodistribution of Magnetic and Luminescent Nanoparticles in Zebrafish Embryos

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In this work, we report the synthesis and physiochemical characterization of magneto-luminescent nanoensembles and their proactive toxicity evaluation in zebrafish (Danio rerio) embryos as a model organism. Their synthesis is accessible through integrating spherical Fe3O4@SiO2 core-shell nanoparticles with Ce3+ and Tb3+ doped GdOF luminophore, concurrently capping in situ with chitosan biopolymer. The bifunctional Fe3O4@SiO2/GdOF:xCe3+,yTb3+ nanoparticles manifested near superparamagnetic behavior at 300 K, simultaneously, display green emission, arising from the characteristic narrow emission lines assigned to the 5D47FJ transitions (J = 6-0) of Tb3+ ion. These nanoparticles exhibited no acute toxicity to zebrafish embryos with-chorions and chorion-off ones up to 100 mg L-1 exposure concentration. Taking the advantage of coexistence of Fe and Gd elements in same nanostructure, their uptake and biodistribution were examined in vivo via Synchrotron X-ray Fluorescence (SXRF) mapping. Strikingly, it was found that magneto-luminescent nanoparticles are highly bioaccumulated in the gastrointestinal tract, revealing more particles uptake through oral exposer and no disintegration of the magnetic and luminescent entities from each other in biological environment. This result demonstrates that SXRF imaging can be an efficient approach to provide more in-depth information on the toxicity of bifunctional nanomaterials, exploiting in vivo the nano-bio interface.

S2-14. Silver nanoparticles effects on Vicia faba: evaluation of cytotoxicity and alteration in photosynthetic activities

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Nowadays, nanoparticles (NPs) are used in several industrial sectors in which silver nanoparticles (AgNPs) are the most commonly applied NPs in commercial product due to their antimicrobial properties. In this context, the growing use of AgNPs and, consequently, the increased release of AgNPs to the environment, it has generated a great concern about the AgNPs effects on ecosystems. The present study aimed to determine the effects of AgNPs in Vicia faba by evaluating the cytotoxicity, genotoxicity, mutagenicity, and alteration in photosynthetic activities. V. faba seeds were germinated in cotton in a BOD incubator, kept at 20 °C and 85 ± 5% humidity.During germination process, the seeds were daily watered with distilled. After germination, the seedlings were divided in three groups and exposed to different treatments. The seedlings were submitted to AgNPs with diameters of (i) 25 nm and (ii) 50 nm at a predetermined concentration of 100 mg.L-1; and (iii) distilled water as the control group. After the treatments, an analysis of cell division at meristems of V. faba roots was executed for the determination of the mitotic indices (MI), cell death (CDI), chromosomal aberrations (CAI), and mutagenicity (MTI). Additional analyses about efficiency of photosystem II were also executed by using a chlorophyll fluorescence imaging system in which it was determined Qymax, NPQ and qN parameters. Finally, a statistical

analysis was performed using Student's T-test ($p \leq 0.05$). The results demonstrated that 25 nm AgNPs were cytotoxic and mutagenic while the 50 nm AgNPs were only genotoxic. The data also revealed changes in the operation of Photosystem II (PSII) induced by the AgNPs of 50 nm, increasing the non-photochemical quenching (NPQ) of PSII. . In summary, our findings indicate that AgNPs was harmful to V. faba, proving to be a potential contaminant to the environment.

S2-15. Metal nanoparticles prepared by laser ablation used as antibacterial surfaces

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In this work we frabicated metal nanoparticles by laser ablation technique in differente liquid media under different energy conditions. We used a Nd: YAG laser system operated at 1064 nm of wavelength with 10 Hz of repetition rate. The structural and optical properties of colloidal samples were characterized by SEM, UV Vis spectroscopy and FTIR. The nanoparticles were deposited over silica and polyehylene substrates, the antibacterial effect was analyzed using colonies of E. Coli and Staphylococcus Aureos, this results show the favorabilty of nanoparticles killing bacterias when this was illuminated with UV-Radiation at 360 nm. We report better behavior as antibacterial surface for TiFeO and ZnFeO nanoparticles prepared at 80 mJ in acetone deposited over polyethylene.These results are favorable for medical applications, usually in surgical devices.

S2-16. Silica Coated Super-paramagnetic Iron Oxide Nanoparticles:A New Generation in Vivo T1 MRI Contrast Agent

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Magnetic resonance imaging (MRI), a sophisticated promising three-dimensional tomographic noninvasive diagnostic technique, has intrinsic advantage in safety when compared with radiotracer and optical imaging modalities. However, MRI contrast agents are less sensitive than complexes used in other imaging techniques and toxicity issue still endures in nanoparticle-based MRI-T1 contrast agents. Therefore, demand for nontoxic novel (T1&T2) T1-weighted MRI potential candidate with ultrasensitive imaging and advanced functionality is very high. In this research, silica coated ultra small monodispersed super-paramagnetic iron oxide nanoparticles were synthesized via thermal decomposition which demonstrated high performance T1-weighted MRI contrast agent for heart, liver, kidney and bladder. Advanced characterization techniques were used to investigate the crystal structure, morphological evaluation, concentration of iron, size distribution, active modes and magnetization of as-synthesized nanoparticles. Transmission electron microscopy (TEM) results have illustrated that the diameter of SPIONPs was in the range of 4nm and the average size of Fe3O4@SiO2 was about 30nm~40nm. X-ray diffraction (XRD) and Raman spectroscopy analyses revealed the purity in phase of the prepared SPIONPs. These magnetite nanoparticles exhibited weak magnetic moment at room temperature because of spin-canting effect which escorted high positive signal enhancement ability. MCF-7 and HeLa
cell viabilities experiments demonstrated good biocompatibility of the SPIONPs. In vivo T1-weighted MR imaging of heart, liver, kidney and bladder after intravenous injection of nanoparticles further verified the high sensitivity and biocompatibility of as-synthesized magnetite nanoparticles. These results reveal silica coated SPIONPs as a promising candidate for T1 contrast agent with extraordinary capability to enhance MR images.

S2-17. Optical properties of Eu 3+ and Tb 3+ - doped tin dioxide nanoparticles prepared by coprecipitation method

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Rare earth (RE) ion doped phosphors have attracted great interest during the past several decades due to their unique physical and chemical properties. RE ions can display many meaningful properties in optics, electronics, and magnetics, originating from f-f electronic transitions within the 4f shell. Doping of wide band gap of metal-oxide semiconductors with RE proved to be a successful tool for tailoring their electrical, optical and microstructural properties. Tin dioxide combines high electrical conductivity with optical transparency. The luminescence of pure SnO2, observed in the UV and/ or visible region (350-550 nm) is generally correlated with the presence of crystalline defects, mainly oxygen vacancies, the most likely candidates for recombination centers in emission processes for SnO2 samples. In this work, the incorporation of the Tb3+ and Eu3+ - doped SnO2 nanoparticles were prepared by coprecipitation method. In semicontuctor nanocrystals, lattice distortions near the surface may produce distinct RE3+ environments, which may be enough to affect their band structure and to relax the dipole-forbidden rule. SnO2:Eu3+ presents the appropriate energetic configuration, which takes effect the luminescence in visible range. The ground state (7F0) and some of the metastable excited levels of Eu3+ ion (5D0, 5D1) are situated in the band gap of SnO2. It allows to observe a typical orange/red emissiom from the excited 5D0 to 7FJ (J=0-4) levels. SnO2: Tb3+ presents the transition 5D4→ 7F6 more intense than 5D4→7F5 and exhibit bluish emission in chromaticity diagram. The CIE parameters and CCT values were also varied and the emission color can be tuned from warm to cool light. In addition, this work opens the possibility to produce new functionalized nanoparticles with control of luminescent properties for photonic and biomedical applications.

S2-18. Photothermal properties of gold-silver hollow nanoparticles on silica optical phantoms

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In this work, we present a study of the photothermal effects of gold-silver (Au-Ag) hollow nanoparticles (HNPs) inside of silica (SiO2) optical phantoms as a diffuse medium for near-infrared (NIR) light. First we fabricate optical phantoms with SiO2 nanospheres embedded into a transparent polyester resin matrix, homogeneously dispersed on it, where the tuning of the optical properties is controlled by changing the concentration of the nanospheres in the medium. The photothermal effect of the hollow Au-Ag HNPs illuminated with NIR light has been already proved by some researches, however in few investigations the photothermal effects are studied on turbid optical phantoms that mimic living tissues. This research shows experimental results of the temporal variation of the superficial temperature in cylindrical and rectangular SiO2 optical phantoms with different Ag-Au HNPs concentration, locations and depths inside the optical phantoms by illuminating with a laser beam operating at a wavelength of 808 nm. In addition, to evaluate the photothermal effects of the system, some thermodynamic parameters as the efficiency of light-heat conversion and the constant of heat dissipation of the Au-Ag HNPs are calculated by using the temporal temperature profiles measured by an infrared camera.

S2-19. Photoacustic Study Of Gold Nanoparticles Aggregates

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Nanosystems have different photophysical and photochemical properties according to composition, sizes and shapes. These properties have allowed the development of nanomedicine as an effective tool in the detection (diagnosis) and phototherapeutic treatment of diseases. Importants studies studies have been developed in this area in the last years. In particular, the use of NS for the therapeutic treatment of neoplastic diseases: photothermal therapy (PTT). In this treatment, the relaxation of the excited state generates a local increase of temperature which is responsible of cancer cell death. Because of this, the study of the photoacoustic (PA) and photothermal properties of different NS is fundamental for their understanding. Previous studies have shown that nanoparticles (NPs) aggregates plasmons have an increase of the electric field in the gap zone. This increase in the field could lead to a localized increase in temperature and, therefore, an increase in the PA signal. At the same time when the aggregate gets larger the electric field diminish. In this sense, little NP aggregates could be efficient drugs for PTT than isolated NP. Also, the bathochromic shift of little aggregates plasmon is an advantage for PTT.In this work we study the UV-visible and near infrared (NIR) spectra for the aggregation process of gold nanoparticles (AuNPs). For the aggregation process different methods were performed. UV-visible, NIR and PA spectroscopy were used to study these systems. Also Transmission Electron Microscopy (TEM) assays and calculations using Mie theory were performed. Experiments shows an increase of PA signal when little aggregates are formed and a diminution of PA signal in presence of larger aggregates. PA spectra (of isolated and differents AuNPs aggregates) are in agreement with absorption spectra calculations and differ from experimental extinction spectra. In this sense, little aggregates could be good candidates for PTT and PA diagnosis.

S2-20. Quantum Yield characterization of β-NaY1-xF4Ybx-yREy (RE = Er, Tm) upconverting nanoparticles

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In an upconversion process multiple photons are absorbed followed by the emission of a single photon with total energy equals to the sum of energy of the previous absorbed photons. Currently, it is well known that NaY1-xF4Ybx-yREy in its hexagonal phase (β -phase) is the most efficient upconverting nanoparticle (UCNP). Moreover, this UCNPs present low toxicity, allows surface modification, and also can have different kind of ligants attached to their surface. Their emission can be tuned to different wavelengths changing the RE elements. Doped with Tm, they can be both excited and having emission within the NIR window, which present deep tissue penetration. These unique properties make them suitable for different biomedical applications, such as tissue staining and optogenetics. NIR is minimally absorbed by water and hemoglobin, thus being easily collected from the body surface. In that way, UCNPs, which can attach to different kinds of cells (e.g. cancer cells), can be excited inside the body by NIR light. Biomedical applications must be precise in terms of dosing bio-markers in order to have the best signal quality and yet avoid overuse. Even though this material is a strong biomarker candidate, there is no standard way to measure its upconverting efficiency. Usually it is measured by comparing the brightness of different samples. With that in mind, this work presents the upconverting efficiency of different commercial UCNPs characterized by means of the Quantum Yield property. This parameter depends on the excitation intensity and is defined as the ratio of the number of photons emitted to photons absorbed. The light emitted was collected by photodiodes and compared to the light emitted from reference dyes, whose properties are well established. The beam profile of the 965 nm laser source was evaluated using a commercial CMOS camera.

S2-21. Redox-responsive Nanoparticles for PpIX-mediated PDT

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Cancer is among the leading causes of mortality worldwide, with 8.8 million related deaths reported in 2015. The efficiency of conventional treatments is hindered by several factors, demanding new approaches or the optimization of current treatments. Photodynamic therapy's (PDT) cytotoxicity is based on the oxidation of cellular components by reactive oxygen species produced when light-sensitive molecules are activated in an oxygen-rich environment. PDT efficiency depends on the photosensitizer buildup at treatment site. Drug delivery is a problem that has been addressed by Nanotechnology through the development of nanocarriers, and combining PDT's oxidative damage to induce cell death with Nanotechnology's targeting ability can optimize cancer treatment. In this work, we verified and compared the efficiency of polysilsesquioxane nanoparticles (NPs) fabricated with protoporphyrin IX (PpIX) against the efficiency of the free PpIX in monolayer cultures of tumor and healthy cells. Results of the PDT effects on the human cell lines of breast carcinoma, non-melanoma skin cancer and keratinocytes in the presence of PpIX-containing silica NPs displayed similar phototoxicity when compared to the free PpIX (with approximately 90% of cell death), with lower cytotoxicity when samples were protected from light. The PEGlated nanostructure, however, yielded higher cell viability when applied in keratinocytes cultures (60% of cell death). To determine the mechanisms underlying the PDT damage in different cell lines, distribution kinetics, type of cell death and differences in the mitochondrial membrane potential will be assessed.

S2-22. Biopolymer nanoparticles for the best transdermal delivery of the 5-aminolevulinic acid.

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The pharmaceutical industry has been developing many nanotechnology systems for both diagnostic and treatment applications for many diseases, including non-melanoma skin cancer. Thus, it is common to use biopolymers in these nanosystems to improve the permeation of drugs by transdermal route and to improve the bioavailability of these drugs. [1] With this objective, nanoparticles (NP) synthesized from the biodegradable polymer poly (lactic-co-glycolic acid) (PLGA) have been developed for potential use in a controlled release system of the prodrug 5-aminolevulinic acid (5-ALA) to improve the topical action of photodynamic therapy (PDT). NP-PLGA-ALA was developed by the double emulsion method followed by the evaporation of the organic solvent and its physical-chemical stability was determined monitoring over time the pH, size and Zeta potential. [2] The initial pH of the NP-PLGA-ALA was (4.66 ± 0.02), with mean size by Dynamic Light Scattering of (469.1 ± 5.2) nm and with Zeta potential of (-4.62 ± 0.07) mV. Subsequently, NP-PLGA-ALA were tested in comparison with free 5-ALA and NP-PLGA for cytotoxicity in human squamous cell carcinoma (A431) and keratinocyte cells. NP-PLGA-ALA increases the bioavailability of 5-ALA, and consequently cytotoxicity leading to cell death. The results may demonstrate the efficacy of this in vitro nanoencapsulated system [3] which may demonstrate an improvement in the use of free ALA in PDT.

S2-23. Boosting Anti-Tumoral Immunity with Porphyrin Lipoprotein Mediated Photodynamic Therapy

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Photodynamic therapy (PDT) is a minimally invasive, clinically approved cancer treatment, in which photosensitizers that have accumulated at the tumor are irradiated to generate reactive oxygen species that induce death of tumor cells. This cell death can trigger immune responses that may delay tumor growth or induce regression of distant, untreated tumors. Accordingly, this immune activation may synergize with immunotherapies, and increase the number of patients who achieve long term remission. Previously, our group developed porphyrin

lipoprotein (PLP), a stable, ~20 nm biomimetic nanoparticle with a hydrophobic core amenable to drug loading, and a porphyrin-lipid monolayer. This nanoparticle can integrate positron emission tomography, fluorescence imaging and PDT onto one platform. Here, we aim to: 1) determine if PLP mediated PDT can induce immune responses that control tumor growth, and 2) boost anti-tumoral immunity by loading Freund's adjuvant into PLP's core to create an imageable nanovaccine capable of dual PDT and immunotherapy. In preliminary studies, dual subcutaneous AE17OVA mesothelioma bearing C57Bl/6 mice were injected with 4 mg/kg of PLP or saline intravenously. Whole body fluorescence imaging revealed that PLP accumulated in tumors 24 hours post injection. Upon laser irradiation (670 nm, 75 J/cm2, 100 mW/cm2), primary tumors in mice initially regressed, whereas primary tumors in control mice continued to grow. In PLP treated mice, the growth of secondary, untreated tumors was delayed relative to control mice. These findings suggest the potential involvement of the immune system. Flow cytometry studies are ongoing to characterize the immune mechanism underlying tumor growth delays after PLP mediated PDT. We will establish the feasibility of a nanoparticle platform that colocalizes an adjuvant with a photosensitizer for combination PDT and immunotherapy, and thereby, help position PDT and immunotherapy as feasible combination treatments.

S2-24. Photosensitive Polymeric Nanocarriers For Intracellular Delivery Of Therapeutic Principles

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Photosensitive nanocarriers (PNcs) offer excellent space-temporal control in therapeutic principles (TP) delivery. This is explained because the PNcs are based on polymers conformed by photoactive groups that may experience structural, conformational and sterochemical changes such as photoisomerization and photocleavage after exposition to ultraviolet light (UV) and near infrared irradiation. Atrial fibrillation (AF) is the most common sustained arrhythmia and their available treatments, as the use of antiarrhythmic TP, are ineffective and hampered by significant side effects. This work reports on the development of a functional photoresponsive polymeric nanocarrier for intracellular TP delivery in cardiac cells. Herein, we used Nile red dye as a model of TP to proof encapsulation/release concept. The new ultraviolet PNcs were readily synthetized by modification of the biopolymer chitosan with a UV-photosensitive molecule. The PNcs were further functionalized with a transmembrane peptide bioreceptor, to achieve the cardiomyocytes site specific TP delivery. The resultant nanobioconjugate (NBc) at 0.15 mg/ml demonstrated to be capable of encapsulating 19.38 μM Nile red and photoreleasing 98 % of this cargo in 8 seconds by ultraviolet exposure, without presenting cellular phototoxicity at the tested time. Fluorescence microscopy experiments showed that the NBc had affinity for the cardiomyocytes, demonstrated by a higher internalization extent, with respect to the concomitant PNcs counterparts. The NBc occupied an average of 18.90 % of the cells area, after 8 h of incubation. Such NBc concentration resulted to be biocompatible as demonstrated by MTT viability experiments. The overall results demonstrated that delivering TP in a controlled temporal space fashion by functional NBc may be a strategy to improve the effectiveness of the highly specific AF treatment, by incrementing intracellular concentration of TP in cardiomyocytes that may decrease their side effects.

S2-25. Brain activation during execution and observation of motor actions in 6-month-old infants: a fNIRS study

Vera Mateus & Ana Osório (Social and Cognitive Neuroscience Lab, Mackenzie Presbyterian University, Brazil)

Understanding others' actions, intentions, and emotions seem to reflect the influence of a mirror neuron system (MNS) (e.g., Rizzolatti & Craighero, 2004). Previous research has shown similar cortical areas activated when adults perform a motor action and when observe the same action being performed by others (e.g., Mukamel et al., 2010). In fact, human beings learn about others' intentions by watching their actions and behaviors, which constitutes a key mechanism of the development of social communication. However, studies investigating this overlapping neural network during observation and execution of motor actions in infants are still very scarce. In this regard, Shimada and Hiraki (2006) showed that, similarly to adults, 6-month-old infants presented activation

in motor areas when performing and when observing another's action. Though, further research should continue to address this issue in infants, specifically the developmental trajectory of the neural processing of observed and performed motor actions, as well as its contribution to infants' subsequent social development. Thus, the present work aims to assess 6-month-old infants' brain activation during the execution and observation of a motor action (i.e., grasping). Functional near-infrared spectroscopy (fNIRS) will be used to assess the hemodynamic response in infants' primary motor córtex (MI), during two phases: the execution phase, in which the infant will be encouraged to grasp an object (a ball), and the observation phase, in which infants will observe the researcher's hand slowly reaching through a curtain, grasping a ball on the table and removing it from the scene. The experimental paradigm will last approximately 12 minutes. Currently, we are finalizing some details regarding our experimental paradigm and the NIRS cap. Thus, we expect to begin data collection next February, so that we may already present some preliminary data at the event.

S2-26. Assessment of neuroplasticity in the human brain with near-infrared spectroscopy

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Rickson Coelho Mesquita, University of Campinas (UNICAMP);

The brain is a complex and dynamical system that is always modifying itself to adapt and evolve. This capability of changing constantly is known as brain plasticity, or neuroplasticity. Although neuroplasticity is pointed as the key cerebral feature to brain reorganization and development, the study of neuroplasticity is restricted to the limitations of current clinical neuroimaging modalities, such as electroencephalography (EEG) and functional magnetic resonance imaging (fMRI). The goal of this project is to employ near-infrared spectroscopy (NIRS) and graph theory to assess neuroplasticity based on hemodynamic spontaneous fluctuations of the brain during the resting state and functional activation. NIRS is a neuroimaging technique with potential to overcome some of the limitations presented by fMRI and EEG due to its high specificity, high temporal resolution and portability. We first attempt to validate and compare NIRS-based functional networks with multimodal methods by combining NIRS with fMRI and EEG. Then, we aim to evaluate effects of neuroplasticity with NIRS in two different scenarios: during rehabilitation of stroke patients and during brain development of infants.

S2-27. Development of a Wearable Near Infrared Spectroscopy System

Giovanni Hering Scavariello (IFGW - UNICAMP), Rickson C. Mesquita (IFGW - UNICAMP)

Around the world, neuroscience is one of the most well-funded research fields. According to the NIH database, it is the fourth health science in terms of total investment. Nowadays, there are many global efforts to understand the human brain, such as the BRAIN Initiative (USA), the Human Brain Project (EU) and BRAINN (Brazil). The uniqueness of the human mind poses a great challenge for its understanding, and so it is desirable that neuroscientists be able to assess different kinds of questions/populations. Two important steps in this direction are the monetary/physical accessibility and the non-invasiveness of the techniques used to answer those questions. The Biomedical Optics Lab (LOB) at UNICAMP has pioneered the development and application of non-invasive and non-ionizing optical neuroimaging in Brazil. Near Infrared Spectroscopy (NIRS) is one of our lab focuses, it is a portable, non-invasive and low-cost neuroimaging tool that allows monitoring of oxy/de-oxy haemoglobins concentration changes and general hemodynamics. The characteristics of NIRS instrumentation enable measurements to be taken at the bedside / at the surgery room and also multimodal measurements with other neuroimaging modalities such as electroencephalography (EEG) or MRI. Despite these advantages, NIRS experimental setups generally involve the use of optical fibres, restraining the subject's mobility. The size and weight of the fibres can also induce motion artefacts or probe coupling issues. In fact, this is a limitaiton imposed by most neuroimaging modalities, save some exceptions such as EEG. Although classic NIRS setups work well at controlled environments or at the bedside, they hamper measurements in real-world environments such as measurements during physical activity, physiotherapy or measuring children. My project aims to develop Brazil's first wearable NIRS system, enabling studies regarding the human mind in real-world conditions.

S2-28. Applications of Diffuse Optical Spectroscopy in experiments of functional neuroscience

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To Control brain activity may represent an improvement in the quality of life of people with some type of disorder, such as attention deficit, autism, cerebral palsy among others. Brain-machine interface (BCI) and neurofeedback are approaches that use brain activity to control a external device (in the case of BCI) or to self-regulate brain activity (in the case of neurofeedback). Monitoring of brain activity is usually done by neuroimaging techniques. Among the different techniques, Diffuse Optical Spectroscopy (DOS) shows to be a promising to perform BCI and neurofeedback due to portability, low cost, temporal resolution among other aspects. This project aims to study and to develop data processing and analysis techniques
DOS for the control of brain activity in BCI systems and Neurofeedback.

S2-29. Cytotoxicity, Genotoxicity, DL-50, post-treatment of neuronal cells with LLLT.

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The neurological diseases, degenerative and traumatic, that affect the central nervous system kill 6.8 million people / year, 12% of global deaths. (source: WHO - 2017). In domestic animals, dogs and cats, there is also an increase in the degenerative processes that affect the central nervous system, affecting 68% of animals with age of 15 years or more. The incidence and diagnosis of these neurological disorders are a direct reflection of the increased longevity of animals and humans, as well as technological advances in the medical field and other sciences that benefit the quality of life. Faced with this, photonic therapies have been studied as a coadjuvant in the treatment of acute, chronic and degenerative neurological conditions. The proposed research project aims to search for data to help establish an effective protocol; with the best interaction of light with nervous tissue; without causing any effect that could alter the physiological functioning of the nerve cell, as to wavelength, fluency, pulse rate, temperature, treatment time and other laser parameters. The study will use a cell line A172 (neuroblastoma), Ar Ga Al diode laser (DMC®), with wavelengths (λ) 830 nm, 40 mW and 250 mW, and (λ) 660 nm, 35 mW, continuous and pulsed emission mode; at the energy densities of 4, 8, 16, 32 and 64 J / cm 2. Verification protocols of cell viability mechanisms, mitochondrial activity, plasma membrane integrity, DNA damage, inhibition or acceleration of the cell cycle, programmed cell death (apoptosis / necrosis); cumulative or late effects and DL-50 of energy supported by the cell in vitro post-treatment with low power laser, are used for this scientific research.

S2-30. Methylene blue based biocompatible molecular contrast agents for pump-probe optical coherence tomography (PP-OCT).

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Atherosclerosis, a condition in which plaque accumulates on the inner wall of arteries, is often recognized as a precursor to cardiovascular diseases (CVDs), the most common causes of death in the US. Optical Coherence Tomography (OCT) is an optical diagnosis tool, which can be used to obtain high-resolution cross-sectional images of atherosclerotic plaque, that enables visualization of tissue microstructure and functional information. However, plaque components, such as foam cells (LDL-loaded macrophages), can be misclassified due to their signal similarities to fibrin accumulations, cholesterol crystals and microcalcifications. To overcome these challenges, we developed a biocompatible contrast agent to enhance molecular imaging of a Pump-Probe OCT (PPOCT) system. Methylene blue (MB) was encapsulated into poly lactic-co-glycolic acid (PLGA) particles by an

emulsion/solvent evaporation technique. Fabrication parameters were controlled to synthesize particles with desired properties such as: size, encapsulation efficiency, degradation rate, and particle surface functionalization. The encapsulation of MB protects it from the enzymatic reduction to leuco-methylene blue (92.8% protection) and reduces the singlet oxygen generation by the excited MB molecules by 78.3%. Likewise, the PLGA shells improve the OCT signal by enhancing the scattering of light. The surface of particles was modified with ligands that can target molecular biomarkers involved in atherosclerotic plaque formation such as vascular cell adhesion molecules (VCAM-1) and apoptotic macrophages. This modification is expected to enhance tissue selectivity, provide detailed information on the local biochemistry and yield visualization of pathological processes. PLGA-based contrast agents were tested in human postmortem artery sections to study particles permeability as a function of particle size and its molecular selectivity.

S2-31. Assessing tissue biomechanics during embryonic development using multimodal imaging combining Optical Coherence Tomography and Brillouin Spectroscopy

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Background: Mechanical forces play a crucial role during embryonic development. Understanding these mechanical changes is important to gain a deep insight into embryogenesis. Due to the sub-optimal measurement techniques, the interplay between tissue forces and stiffness remains poorly understood. This shows that there is a need of non-invasive, non-contact imaging that can quantify the stiffness of the embryonic tissues in-situ with high resolution. Methods: A novel multimodal system combining Optical Coherence Tomography and Brillouin spectroscopy is used to map the elastic modulus of the tissue of the mice embryos. Results: The biomechanical properties of the neural tube region of the mice embryos at embryonic day E 8.5 and E 9.5 (n=2) is acquired and mapped with submicron spatial resolution. We showed the variation of elastic modulus in the neural tube region at different embryonic developmental stages using the noninvasive, noncontact multimodal imaging technique with a high spatial resolution and sensitivity. Conclusions: This work shows the potential use of the multimodal imaging using Optical Coherence Tomography and Brillouin spectroscopy to study the biomechanical properties involved in the embryonic development in-situ. This technique can be significantly used to show the changes in the elastic modulus of the tissue during neural tube closure.

S2-32. Automated detection of infiltrated-cancer margins using Optical Coherence Tomography

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Purpose: In glioma surgery, it is critical to maximize cancer resection without compromising adjacent healthy, non-cancerous regions. Optical Coherence Tomography (OCT) is a non-invasive, label-free, cost-effective technique capable of imaging tissue in three-dimensions and real time. In this study, we have developed a novel mathematical model known as the Blind End-Member and Abundances Extraction method (BEAE). This constrained optimization technique was used for quantitative evaluation and feature extraction over the OCT datasets for automated cancer detection in image-guided surgery. Experimental Design: Volumetric OCT datasets were obtained from 21 glioma patients at Johns Hopkins using fresh ex vivo human brain tissue specimens. Within the training set (12 patients), a logistic regression classifier was used to predict each OCT A-line as either cancerous or non-cancerous. Then, a diagnostic threshold (i.e. % A-lines required to classify an OCT volume as cancerous) was determined using a leave-one-patient-out cross validation and receiver-operating characteristic (ROC) curve. Finally, a non-labeled independent dataset (9 patients) was used to perform an extra blind

validation to corroborate the sensitivity and specificity of our model. Results: A diagnostic threshold of 80% was chosen to maximize cancer resection while maintaining maximum healthy tissue based on the ROC analysis. From which, we were able to achieve excellent sensitivity (99.15%) and specificity (86.21%) in brain cancer diagnosis. Conclusions: We present a novel mathematic model for automated brain cancer detection based on OCT datasets. The BEAE method is a promising tool which may facilitate fast and reliable detection of brain cancer within the operating room.

S2-33. In Utero Optical Coherence Tomography to Evaluate Changes in the Murine Fetal Brain Vasculature Due to Prenatal Alcohol and Cannabinoid Exposure

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Prenatal substance abuse is one of the main causes of birth defects in the United States. The severity of the defect depends on the substance, amount used, and the period of gestation during which the abuse happened. Many women continue their substance abuse well into the second trimester of their pregnancy, which is considered the peak period for fetal neurogenesis and angiogenesis. Although several studies have documented morphological and behavioral changes due to maternal substance abuse, there is far less research focused on the acute vasculature changes in the fetal brain. In this study we use optical coherence tomography (OCT) to evaluate changes in fetal brain vasculature, in utero, minutes after maternal alcohol and cannabinoid exposure respectively. Ninety-five percent ethanol, diluted in water (water was used for the sham experiments) at a dose of 3g/kg, was administered via intragastric gavage to the pregnant mice after initial (i.e. pre-ethanol) OCT imaging of the embryonic brain. Subsequent measurements were taken every 5 minutes for 45 minutes. CP-55,940, an often-used synthetic cannabinoid in research, was sprayed on the liver of the pregnant mother at a dose of 2 mg/kg after initial OCT measurements. The cannabinoid was suspended in a solution of DMSO: Alkamuls El620 (Rhodia, NJ): lactated Ringer's (1:1:18), and this solution was used for the sham experiments. Subsequent imaging was performed similar to the alcohol experiments. Results showed a rapid and significant decrease in vessel diameter as compared to the respective sham groups. This preliminary data showed that maternal alcohol or cannabinoid exposure, results in immediate and significant vasoconstriction in the fetal brain.

S2-34. Epidermis optical attenuation coefficient modulation at arterial pulsation rate

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Video plethysmography (vPPG) is a noninvasive, remote diagnostics method that monitors blood pulsation by measuring subtle time-variation of the skin reflection coefficient. However, the mechanism responsible for the fluctuation of the skin color at heart rate, especially for green and blue wavelengths, is not well understood. Our hypothesis was that skin optical properties are modulated by arterial transmural pressure propagation, affecting all skin layers and all wavelengths where skin inhomogeneities are relevant for light propagation properties. Using optical coherence tomography (OCT), we show that the optical attenuation coefficient of the outermost layer of the epidermis is modulated at heart rate, being the modulation surrogate for the mechanical changes in the skin due to blood pulsation. We propose a hydraulic shock hypothesis to illustrate the phenomenon. The results explain vPPG results with blue light, that is limited to non-vascularized regions of skin.

S2-35. Design of a portable and low-cost spectrometer for analysis of forest fuel

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Roberto Carlos Barragán Campos - University of Guadalajara
Guillermo García Torales - University of Guadalajara

In fields related with biological areas, as botanist, ecologist, agronomist and others, the interaction of electromagnetic radiation with vegetation is too relevant to know the biochemical characteristics of the forest and crops. In the forest, is necessary to know the amount of resins, oils or waxes of several plant species to prevent and to detect phytosanitary problems. Exist different spectroscopic methods used in this field, and one of the most common is to use a white light source, and use gratings or prisms to diffract or disperse the light, but to use these elements can raise the device's cost. Our objective in this work is design a portable and low-cost spectrometer, able to measure the transmittance of tree leaves and calculate the absorbance, in three different bands of the electromagnetic spectrum, performing the measures in situ, avoiding to cut the sample. An area of the sample is illuminated by a RGB LED, in a sequence of three different wavelengths. The optical system is composed by two lenses that control the spot size, and the emission angle, and collimate it. The detector is composed by a semi-sphere with digital optical sensors placed equidistantly. The sensors send a frequency signal to a microcontroller and the data is saved on an external memory, through I2C communication. Is programmed a graphical interface in a PC, and through it the data inside the external memory is exported to a text document and later processed with Scilab to obtain the absorbance with the transmittance measures. The obtained results will be compared with the measures of a commercial spectrophotometer.

S2-36. Combat the Bovine Tick by Photodynamic Therapy

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The livestock sector has great importance in the economy. However, the losses caused by tick parasitism in cattle are numerous, being considered the most impacted species in dairy and beef cattle. One of the most used strategies for ticks elimination is the carrapatient, that can lead to the breakdown of the immunological stability of the cattle, besides damages to the environment. An alternative to the solution to this problem is to use photodynamic therapy (PDT) to combat the bovine tick. The technique consists of the interaction of a photosensitizing (PS), light and molecular oxygen, which promote the formation of reactive oxygen species, inducing cell death. The aim of this study is to develop a protocol to combat bovine tick using PDT and to compare the results obtained with commercially available carrapatients. Five groups will be evaluated: control, light, PS, PDT and carrapatient. The chosen PS is curcumin and the light source is sunlight. For the pilot study, ten tick eggs and 10 tick larvae will be placed in petri dishes, incubated in curcumin at 40 µg/mL for 3 hours and then exposed to sunlight for 12 and 24 hours in order to evaluate the concentration parameters of curcumin and optimal irradiation time. All experiments will be performed in triplicate. The eggs are obtained from the hatching of the teleóginas previously collected and the larvae originate from the hatching of the eggs remaining. A difficulty faced in this study is to feed the larvae in order to keep them alive after the hatching of the eggs. The results obtained will help to establish the most effective strategy for using curcumin as a substance to assist in the fight against ticks, contributing to the safety and welfare of animals and humans, once carrapatients are harmful to the health of both.

S2-37. Evaluation of photonic techniques for decontamination of beef.

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Introduction: Microbial contamination of meat is a public and economic health problem. Food products of animal origin, in particular beef, are contaminated before, during and after slaughter. Contamination by pathogenic microbial agents can promote changes in their nutritional value, sensory characteristics, and disease.

Interventions used for the surface decontamination of meat are not capable of completely eliminating the bacterial load, and are also processes that generate residual substances that can modify the appearance of the product. That is why new technologies are being studied to decontaminate fresh meat and do not cause damage to the environment and ensuring food safety. Objective: decontaminate meat surfaces using fotonics methods as UV-C Light from the development of a new prototype Methodology: The sample used was beef bought from commercial butchers. The collection protocol for the microbiological tests was by homogenizing the meat samples for 1 min with 10 ml of diluent (in the case "PBS" Phosphate buffered saline) for the removal of the bacteria. The size of each sample was 10 cm2 and thickness of approximately 2 mm. For the tests the exposure of light uv-c in different times 1, 5, 10, 15, 20, 25 and 30 minutes were used. Results: The results indicated that light with uv-c decontamination showed a microbial reduction of approximately 1 Log (cfu / cm2) and low stability of the microorganism in the meat.

S2-38. Development of photosensitizing diamond nanoparticles for application in Melanoma Photodynamic Therapy

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Diamond Nanoparticles (NDs) are a class of carbon-based nanomaterials of increasing interest in science and technology. The applications for NDs are being discovered such as biomedical applications as for example: drug delivery. The National Cancer Institute (Inca) estimated approximately 165.580 cases of skin cancer in Brazil in its latest report (2018). Estimates reveal that the impact of neoplasms will increase dramatically in coming decades. Thus, the objective of this study will be to develop nanoparticles from laser ablation and with antitumor activity. NPs act as transporters and drug-releasing agents, protecting drugs from interference of the external environment to reach their target treatment in its active form. The present study proposes the development of photosensitizing nanoparticles (FTs) based on NDs for the application in metastatic melanoma therapy. The results showed that the laser ablation process reduced CVD particle size. The mean hydrodynamic diameter in aqueous suspension after the centrifugation changed from 54 nm. The high stability of aqueous suspension of CVD NDs was indicated by the low polidispersity index (0,2) and a small increase in the mean value of hydrodynamic diameter during the observed period (D = 215 nm). The high stability was provided by the high charge density on NDs surface as suggested by the high value of Zeta-potential (-36.39 and -30.94 mV). The cytotoxic activity will showed 60% and 80% of cell viability against the murino metastatic melanoma B16-F10 cell after 24h, 48h, 72h and 96h of incubation with NDs. Later the NDs will be activated with photoactive molecules and tumor-homing peptides. The antiangiogenic assay using two different endothelial in order to evaluate the nanoparticles and their interaction with the endothelium. The high value of cell viability is an indicative of the cytocompatibility of NDs, indicating the potential use of NDs in biomedical applications such as drug delivery platforms.

S2-39. Using near infrared spectroscopy techniques by identify biomarkers of atherosclerosis of carotid from brain hemodynamic.

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The atherosclerosis of carotid (AOC) is a vascular disease that affects the carotid artery and causes changes in cerebral hemodynamic function. The partial or total occlusions by the presence of fat plaques in the carotid artery causes at least 15-20% of all ischemia events in elderly population without any previous symptoms. Our hypothesis is that the brain hemodynamics is directly affected by the presence of the carotid plaque leading to a state of permanent vasodilatory response to meet metabolic requirements. Currently, there are not robust biomarkers to monitor continuously the brain hemodynamic in the patient, therefore, finding biomarkers related to

AOC could decrease the percentages of ischemic events. The Near Infrared Spectroscopy (NIRS) is an optical noninvasively optical technique with high sensibility to tissue hemodynamics at the microvascular level and with high portability and low cost that allows implementation at border of the bed. The interaction in vivo between biological tissue and infrared light allows infer the concentration changes of oxy- hemoglobin and deoxy-hemoglobin, which makes it a potential tool for assessing the cortical consequences of the atherosclerosis of carotid. In this work, we aimed to translate NIRS methods to clinical monitoring of patients diagnosed with AOC by identifying biomarkers from the brain that relate to the level of stenosis of patient.

SESSION 3 - 28 March, 2019 - 4PM

S3-01. Development of a technique combining photobiomodulation and radiotherapy to enhance the tumoral response to ionizing radiation

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Photobiomodulation (PBM), a technique that is being used for over 40 years, is based on applying light in order to modify cellular processes such as proliferation, tissue regeneration and analgesia. These effects are strong evidences that PBM can modulate the cell cycle and increase tissue oxygenation, which are determinant factors in a radiotherapy result Therefore, in this project it is proposed the investigation of the combination of these techniques to the deleopment of a protocol that enhances tumor damage and treatment efficacy. In vitro experiments will enable the study of PBM in cell proliferation and cell cycle and its combination with radiotherapy, using the MTT assay and flow cytometry. From these experiments it will be determined the protocols to be analyzed in vivo. It will be used an oral cavity cancer model, using the human squamous cell carcinoma cells SCC-25, and in vivo tumor induction will be performed in nude mice. The aim of this experiments is the evaluation of the techniques combination in a model of higher biological complexity and the analysis of tumor damage and animal survival in order to propose a protocol that enhances the cancer response to ionizing radiation.

S3-02. The synergy of technologies in the treatment of chronic diseases

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Chronic diseases such as fibromyalgia and osteoarthritis are increasingly present in the population. The first, Fibromyalgia, is a chronic disease characterized by high intensity pain with non-articular bases, affecting women and reaching between 3% and 10% of the world population. Fibromyalgia affects the quality of life of patients and is currently treated with anti-inflammatory and analgesics that facilitate pain crises. On the other hand, osteoarthrosis is a degenerative disease that predominantly affects seniors, being characterized by chronic and closed pain, around 50% of the elderly population. Therapeutic approaches to pain relief, ultrasound and photobiomodulation have been widely used as a complementary pharmacological treatment of both as diseases. In our research group (São Carlos Institute of Physics, University of São Paulo), the development of new technologies is aimed at the treatment of the most diverse diseases. In this way, the first equipment capable of conjugate emitting the ultrasound and low intensity laser was developed, thus allowing the synergic action of the cavitational effect and the photobiomodulation. Thus, in the last year several manuscripts were published by attending to hundreds of patients affected by the chronic diseases mentioned above. The results point to the improvement of the quality of life of fibromyalgic patients, showing not only the marked reduction of incapacitating pain, but also the restoration of the daily activity condition. In the same way, the patients affected by osteoarthrosis showed not only the marked reduction of pain, but also the increase of articular functionality, providing a return to activities such as crafts, dancing and walking. Thus, through the synergistic action of technologies, the improvement of the quality of the patients has been observed, a fact that is essential for a society, such as the Brazilian, that increasingly aging, drastically altering its age pyramid.

S3-03. Protocol for aPDT, with a biofilm remover and a multiple wavelength diode laser, for the treatment of periodontitis

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Objective:The effects of LLLT on the reduction of the inflammatory process on the gingival tissue of diabetic patients and its systemic effect, is to be determined, by comparing the use of a LLLT, utilizing a multiple laser wavelengths probe, a single wavelength probe and a control group (sham light). All with the same incident doses.Methods:The study is to be performed using a specially developed multi-wavelengths laser cluster probe, with 532, 633, 810, and 980 nm diode lasers and a single 633 nm at incident dose of 10 J/cm2. These parameters were chosen, as it was found to have positive results in a previous study, with those single wavelengths. (Farouk A. et all, 17 May 2007 , Photomedicine and Laser Surgery vol 25, n. 2).
A blood sample of the patient will be taken, previously to the treatment and after. The inflammatory markers with be measured and compared, before and after the treatment is introduced.The comparison will be made, by investigating markers of inflammation on the control group and the LLLT treated diabetic patients with periodontal disease, via blood analysis for glycated haemoglobin, (HbA1c), high□sensitivity C reactive protein, (hsCRP) and lipid profile comprising total cholesterol, low□density lipoprotein cholesterol (LDL chol), high□density lipoprotein cholesterol (HDL chol) and triglycerides.A previous study, investigating the relationship between markers of metabolic control and inflammation in periodontal disease patients with diabetes, reported that he level of glycemic control as measured by HbA1c emerged as the most consistent risk factor associated with the extent and severity of periodontal disease L.P.Lim et al, 2016, https://doi.org/10.1111/j.1600-051X.2006.01032.x

S3-04.Could Hands Be A New Treatment To Fibromyalgia? A Pilot Study

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Fibromyalgia is a chronic disease and presents generalized pain over 3 months after stress, trauma or even infections, the etiology is not well defined, but it affects 3% to 10% of adult women. There are several treatments, but photodynamic therapy has been increasingly emphasized, especially the action of ultrasound and the laser for analgesia and anti-inflammatory action. A recent study reported that patients with fibromyalgia had increased nerve endings around blood capillaries in the palms of patients with fibromyalgia. Therefore, this work was used two innovations, first use of prototype that makes the combination of ultrasound and laser conjugated (Ultralaser) developed by the Laboratory of Technological Support (LAB) of the Physics Institute of São Carlos (IFSC), and then the comparative analysis of the treatment performed in pain points (tender points in the trapezius muscle) and the application in the palms of the hands. Two groups were divided to define the treatment site and each group subdivided into 3, receiving consequently Wavelength of 660nm laser treatment, therapeutic ultrasound, and ultralaser with the same parameters. The patients were evaluated with a questionnaire to the Fibromyalgia Impact Questionnaire (FIQ) before and after treatment and Visual Analogue Scale (VAS) before, during and after treatment.In view of this work, it was observed that the treatment performed on the palms of the hands improved the relaxation of the deep musculature due to the adequate stimulation of the point indicated by the literature, balancing the oxygenation and the circulation obtaining a systemic response. This pilot study concluded that the treatment technique in the palms of the hands significantly surprised the improvement of patients' quality of life, reducing fatigue, sleep quality in relation to the treatment technique in the tender points in the trapezius muscle and the combined action of ultrasound and laser had a superior action.

S3-05. Microbiological and clinical effects of photodynamic therapy as an adjunct to scaling and root planing of chronic periodontitis

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Antimicrobial photodynamic therapy (aPDT) is increasingly being explored for treatment of periodontitis. Here, we investigated the effect of aPDT on human dental plaque bacteria in suspensions and biofilms in vitro using methylene blue (MB)-loaded poly(lactic-co-glycolic) (PLGA) nanoparticles (MB-NP) and red light (660 nm). The effect of MB-NP-based aPDT was also evaluated in a clinical pilot study with 10 adult human subjects with chronic periodontitis. Dental plaque samples from human subjects were exposed to aPDT - in planktonic and biofilm phase - with MB or MB-NP (25 μg/mL) at 20 J/cm² in vitro. Patients were treated either with ultrasonic scaling and scaling and root planing (SRP) or ultrasonic scaling + SRP + aPDT with MB-NP (25 μg/mL and 20 J/cm²) in a split-mouth design. In biofilms, MB-NP eliminated approximately 25% more bacteria than free MB. The clinical study demonstrated the safety of aPDT. Both groups showed similar improvements of clinical parameters 1 month following treatments. However, at 3 months ultrasonic SRP + aPDT showed a greater effect (28.82%) on gingival bleeding index (GBI) compared to ultrasonic SRP. The utilization of PLGA nanoparticles encapsulated with MB may be a promising adjunct in antimicrobial periodontal treatment.

S3-06. Evaluation of photodynamic therapy with methylene blue in Leishmania amastigotes

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Cutaneous leishmaniasis is an infectious disease caused by protozoa of the genus Leishmania. It is a neglected disease whose treatment is extremely aggressive and toxic to the patient. The side effects of conventional treatments include nausea, vomiting, myalgia, electrocardiographic changes, making treatment difficult for patients with heart and pregnancy problems. Photodynamic therapy is an alternative treatment that has been studied, due to the possibility of treat the lesion, locally, which would eliminate the problem of systemic applications and reducing the side effects. In the present study, Methylene Blue (MB) was used as a photosensitizer to evaluate the effect of Photodynamic Therapy on Leishmania amastigotes in co-cultivation with macrophages, strains of L. major and L. braziliensis were used. For this, concentrations of 500, 250, 125, 62.5 µg/ml and irradiated with energy density of 10 J/cm² were used. The macrophages were seeded in 24-well plates and incubated with protozoa in the ratio of 10 to 1 to ensure infection. After infection of the cells the medium was withdrawn, replaced with MB at the concentrations tested, and incubated at 37 ° C for 1 hour. The methylene blue was then removed and replaced with PBS, and then the PDT protocol was performed. After 18 hours, mitochondrial activity and viability were evaluated. The result of the treatment was evaluated by the observation of the mitochondrial activity by the MTT test, and viability, with the Tripan Blue test. It was observed that the interaction with MB significantly reduced the mitochondrial activity of cells even before irradiation, although in the dark there was only toxicity at the two highest concentrations. PDT, on the other hand, was shown to be effective, eliminating 100% of cells after treatment. However, further studies are needed to determine whether protozoa are resistant to PDT when macrophages are killed after PDT, and whether they have potential for re-infection.

S3-07. Antimicrobial Photodynamic Therapy Using Chlorin e6 for Periodontal Disease Treatment

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The general aim of the present study is to evaluate the effect of aPDT using chlorin e6 as photosensitizer (PS) for the treatment of periodontal disease, in vitro and in vivo. In the in vitro study, single and multispecies biofilms will be treated with several protocols, including positive (chlorhexidine) and negative (PBS) control, PS only, light only, and groups treated with aPDT (PS + Light). Different concentrations of chlorin e6, two wavelengths (blue and red) and two doses of energy (15 e 50 J/cm2) will be tested. In single biofilms, the analysis will be carried out by counting colony-forming units (CFU/mL). The multispecies biofilm will be evaluated using qPCR. Confocal microscopy analysis will be performed to determine microorganisms viability maintenance and adherence after treatment of the biofilms. In the randomized controlled split-mouth clinical trial, twenty patients with chronic periodontitis presenting at least two teeth with probing depth (PD) > 6mm in two distinct contralateral quadrants will be selected. The quadrants will be randomly assigned to the control and test groups. After full-mouth SRP, repeated applications of aPDT will be carried out in the test group (immediately after SRP, and after 1, 2, 7 and 14 days after SRP). The protocol that demonstrate better results in the in vitro experiment will be used for aPDT application. Clinical parameters will be evaluated and subgingival biofilm and gingival crevicular fluid samples will be collected for comparison between the control and the test groups in different periods. Microbiological analysis will be performed by counting colony-forming units (CFU/mL) and microbiome analysis by gene sequencing. Multiplex immunoassay will be used to quantify inflammatory cytokines in the gingival crevicular fluid. Statistical analyzes will considered a significance level of 5%.

S3-08. Biophotonic strategy for screening of attractive biotechnology products and properties of bacteria

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Currently bacterial biotechnology industry is critical for the production of many different essential products: ferments, antibiotic, proteins, fungicides, vaccines etc. Furthermore, bacteria remain the main source of new promising products and properties due to their diversity and variability. Microbial biotechnology can change and improve traditional industry systems such as metallurgy due to bioprocessing and biorecovery of critical metals in particular rare earth elements. However, detection of new attractive metabolites and properties of bacteria are limited due to imperfection, high cost and laboriousness of modern screening programs. Furthermore, current detection strategies often require sophisticated instruments not often available in laboratories with fewer resources and it makes high-income countries the biotechnology monopolists.The main aim of the study is creating of screening methods for detection of biorecovery of rare earth elements (Scandium, Yttrium etc.) by Actinobacteria with simultaneous activation of cryptic genes of new antibacterial metabolites especially new effective antibiotics against multi-resistant bacterial strains. The optimal physicochemical conditions, such as pH, ions concentration, the time of cultivation were established in bacteria culture systems. UV-Vis spectrophotometer was used for bioassay measurements. Standard screening and microbiological methods were used for results confirmation. The results show that developed strategy has several advantages compare to classic screening methods due to quick complexes analysis of bacteria strains and changing of different parameters for detection needs.Therefore, the innovative screening methods for detection of biorecovery of rare earth elements by Actinobacteria with simultaneous activation of cryptic genes of new antibacterial metabolites can be applied in global screening programs of new promising products and properties of bacteria.

S3-09. Evaluation of the effects of Photodynamic Therapy with Photodithazine in 9L / LacZ cells.

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Gliosarcoma (GS) is a rare and aggressive tumor of poor prognosis treated by conventional therapies that do not allow the complete elimination of the tumor and produce several side effects. Photodynamic Therapy (PDT) is an alternative treatment that combines three main components: photosensitizer, light and molecular oxygen, causing death in the target cell. The objective of this study was to evaluate the effects of PDT on Photodithazine (PDZ) in GS (9L / LacZ) cells at concentrations of 200 μg/ml and 3.1 μg/ml with 1 h incubation and irradiation at 660 nm at the dose of 10 J/cm². In the internalization it was observed that, in both concentrations tested, the PDZ was internalized by the cells accumulating in the cytoplasmic region and bordering the peripheral region of the nucleus. The trypan blue exclusion test demonstrated viability ≥ 89.8% for groups kept in the dark and control of the clear group. Concentrations of 200 μg/ml and 3.1 μg/ml presented, respectively, 99.6% and 81% death in the PDT group. The MTT mitochondrial activity test showed 100% activity in the control of the light and dark groups and the TFD group obtained reduction ≥ 89.7% of the mitochondrial activity. Thus, PDT + PDZ produced significant cytotoxic effects at both concentrations tested in GS cells in an in vitro study. In order to improve the use of PDT + PDZ, new tests will be performed with cytoplasmic markers and cytotoxicity pathways.

S3-10. Evaluation of photodynamic efficacy against acne monospecies biofilms

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Biofilms are communities of microorganisms that have a high degree of organization with cells enveloped in a self-produced extracellular polymer matrix. Literature shows that approximately 80% of all fungal and bacterial infections in humans are associated with biofilm formation. The microorganisms organized in this way are resistant to treatment with antibiotics and are related to the recurrence of infections. Thus, treatments against microbial biofilms are highly desirable. The photodynamic therapy (PDT) action has shown to be efficient and promising on clinically relevant biofilms. The mechanism of action of PDT occurs when photosensitizer (PS) is

excited by a light source of specific wavelength. This PS can react with biomolecules transferring charge to give rise to radicals and radical ions which will react with molecular oxygen, formed reactive species of oxygen, such as hydrogen peroxide, hydroxyl radicals and hydrogen peroxide (type I reaction). Singlet oxygen formation can also occur when the PS in the excited triplet state transfers energy directly to the oxygen in the ground state (type II reaction). In this context, the objective of this study was to evaluate in vitro the action of chlorine-e6-mediated PDT using LED 660 nm against biofilms of Cutibacterium acnes (ATCC 6919), the main microorganism associated with the etiology of acne vulgaris. Biofilms were formed into 96-well plates (~1x10 8 cells / mL) containing an agar base for 7 days. On the seventh day, the samples were incubated for 15 minutes with the PS at 150 µg /mL and irradiated with 200 J / cm2. Subsequently the samples were scraped, serially diluted and seeded into reinforced clostridial agar. The colony forming units were counted after 24-48 hours. Preliminary results showed a reduction of 2.81 log 10 counts of colony units when applied to PDT. It was possible to conclude with this study that the parameters used were able to significantly reduce C. acnes in the form of biofilms.

S3-11. Influence of formulation in uptake of photosensitizer by bacteria

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Background and Objective: Natural curcumin is a photosensitizer composed of curcumin (CUR), desmethoxycurcumin (DMC) and bisdesmethoxyccurcumin (BDMC). The structural characteristics confer the curcumin lipophilic character (Log P = 2.5) highly soluble in organic solvent such as dimethyl sulfoxide (DMSO). This solvents have high toxicity in cells with unavailable use for clinical formulation. Sucrose may help the solubilization and stability of natural curcumin which is a formulation to be used in clinical applications, as a treatment for throat infection. In this work we investigated the absorbance and interactions of natural curcumin in formulation of syrup (watter + sucrose) in of bacteria of the genus Streptococcus, an oral pathogens.Methods Microbial solutions were incubated with a natural curcumin at 37 ° C in formulations: 1) syrup (water + sucrose) 2) solution alcohol + DMSO. It was centrifuged and supernatant collected for absorbance analysis. The results were obtained correlating the absorbance of the supernatant to the absorbance of the default concentration[2]. In order to justify the result, a study of microbial metabolism by growth curve was carried out. Results and Discussion It was verified that the incorporation of syrup is superior to the solution alcohol/DMSO of three microorganisms studied. The mean percentage of curcumin incorporation of S. mutans was 24% in syrup and 10% in alcohol/DMSO.This difference of absorption is explained due to increase of microbial metabolism with the presence of carbohydrate in solution. Conclusions: The incorporation of photosensitizers by microorganisms is fundamental for efficiency of antimicrobial photodynamic therapy. In addition to the physicochemical characteristics of the molecule the rate of incorporation can be modulated with the formulation. Enabling an alternative strategy for implementing a clinical treatment using curcumin and photosensitizers soluble in toxic solvents.

S3-12. Photodynamic Inactivation of Bacterial Pneumonia using Nebulization of the Photosensitizer and Extracorporeal Illumination

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Pneumonia is one of the largest causes of death worldwide, and it is mainly caused by bacteria. Due to the difficulty in the development and approval of new antibiotics, and the speed in which resistant strains appear, the search for treatments that are effective, safe, and to which there is no resistance becomes urgent. The photodynamic inactivation (PDI) of microorganisms presents itself as an excellent alternative, since there has not been any description of development of resistance to it since its discovery in 1900. Over the past 5 years, our research group has studied the applicability of PDI in the treatment of bacterial pneumonia, using the photosensitizer indocyanine green (ICG) and extracorporeal activation with infrared light. So far, we have demonstrated the principle in vitro and in a murine model, established a pulmonary delivery method for ICV that is suitable for clinical use, and showed the extracorporeal delivery of light in bigger animals. This project proposes to

give the next steps required for the protocol to become a clinical treatment, starting from the optimization of the parameters in the murine model.

S3-13. The association of ultrasound and antimicrobial Photodynamic Therapy for the inactivation of Staphylococcus aureus

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Antimicrobial Photodynamic Therapy (aPDT) has been investigated as an alternative method for the inactivation of microorganisms. This treatment, which is based on the application of a photosensitizer and visible light, has a reduced effectiveness when the microorganisms are organized as biofilm. Recently, Sonodynamic Therapy (SDT) has also been suggested as an antimicrobial treatment and has the advantage of activating photosensitizer by the use of ultrasound (US), which propagates deeper into the tissue and is able to disrupt the biofilm. Thus, this study aimed to associate US with aPDT mediated by curcumin (Cur), in order to disrupt Staphylococcus aureus biofilms and increase the inactivation of the bacteria. For this, standardized suspensions of S. aureus were prepared (109) and after 48 h of biofilm formation, samples received the following treatments: aPDT (Cur and blue LED light), SDT (Cur and US) and SPDT (incubation with Cur and, then, simultaneously application of US and light). Additional samples received Cur, light or US only, or no treatment (control). To determine cell survival, the biofilms were removed and aliquots were serially diluted and plated on Brain Heart Infusion Agar. After 24 h of incubation at 37°C, the colony forming units were calculated. The preliminary results demonstrated that US in combination with aPDT (SPDT) showed higher and significant bacteria reduction compared to the application of SDT and aPDT. Cur, LED light or US alone did not have any effect. This result highlights the enhanced effect of ultrasound and aPDT against S. aureus biofilms.

S3-14. Evaluation of optical techniques for microbiological inactivation of blood

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Blood can be the target of bacterial, viral and parasitic contamination, which can trigger serious diseases. In this study, photodynamic inactivation and ultraviolet radiation were evaluated in the in vitro decontamination of whole blood, erythrocytes, and platelet-rich plasma with S. aureus. For PDI, Photogem® and 630nm light were evaluated, and risks of toxicity of the treatment were determined by hemolysis and cell viability assays. The reductions of S. aureus in whole blood, erythrocytes, and platelet-rich plasma at 15 J/cm2 and 50 µg/mL porphyrin were 1.0 log, 1.3 logs and 0.4 log CFU/mL, respectively. Hemolysis rate for erythrocytes in whole blood was 10.7%. However, erythrocytes hemolysis was 100% when in the absence of plasma. The cell viability assay showed 14% apoptosis rates in isolated erythrocytes, indicating damaging action of PDI, and no damage in platelet. For UVC radiation (254nm), different light doses were analyzed, and the cell viability assay determined the toxicity of technique. The reductions of S. aureus in whole blood, erythrocytes and platelet-rich plasma at 23 J/cm2 were 1.7 logs, 1.1 logs and 2.5 logs CFU/mL, respectively. Relatively small differences were observed in plasma as a function of irradiation time, suggesting some degradation of plasma proteins with 23 J/cm2. The cell viability assay showed normal rates for erythrocytes, however, in the platelets, a high apoptosis rate was observed (74%). Therefore, the optical techniques showed opposite damage effects in each blood component, and the use of one or another technique should be evaluated considering the better microbial inactivation and blood components preservation conditions.

S3-15. Antimicrobial Photodynamic Therapy Challenges Microbial Drug-Resistance

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Background: The rising challenge of microbial resistance to chemotherapy demands immediate implementation of global policies and therapeutic alternatives. Antimicrobial photodynamic therapy (APDT) combines the

administration of a photosensitizer (PS) compound with low-intensity monochromatic light to induce photochemical reactions that yield high amounts of reactive oxygen species (ROS). Since some PS molecular frameworks can be selectively incorporated by pathogens and ROS react with virtually all biomolecules, APDT offers a powerful strategy to challenge microbial resistance of local infections. Methods: In this study we assayed the APDT efficacy, using methylene blue (MB) as PS and red light provided by LED, against planktonic suspensions of high-risk representative fungal and bacterial species. The species tested include A. baumannii (OXA-23 and 143), E. aerogenes (NDM-1), E. faecalis (VAN-B), E. faecium (VAN-A), E. coli (MCR-1, CTX-M8 and 15), K. pneumoniae (KPC-2, IMP-1, OXA-48), S. aureus (MRSA, VISA), P. aeruginosa (VIM-1, SPM-1, GES-5), C. albicans and C. neoformans. For all species, we tested standard control strains compared to azole-resistant yeast, or bacteria resistant to nearly all commercially available antimicrobials, in attempt to observe any cross-resistance in between APDT and standard chemotherapy. Results: More than 5log10 reduction was observed within less than a minute of illumination for non-capsulated bacteria and within less than 5 minutes for yeast and capsulated bacteria. Regardless of resistance phenotype MB-APDT presented species-specific dose-response kinetics suggesting that similar therapeutic protocols may bring successful outcomes in clinical practice. Conclusions: Our study proposes that MB-APDT can efficiently inactivate a broad-spectrum of drug-resistant microorganisms and impair drug-resistance genes selection and dissemination.

S3-16. Photodynamic inactivation using curcumin applying in biofilm developed at polymeric surfaces.

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Infectious disease is a continuous treatment challenge for the hospitals. Ventilator Associated Pneumonia (VAP) is one of the most dangerous respiratory disease, because can lead the patient to death. The majority of the antibiotics can not destroy the cells from the developed biofilm at a surface of the endotracheal tube. The endotracheal tube is a tube which auxiliated the patient to breath. The biofilm is a way of the bacteria to grow, formed by the cells and a matrix called extra polymeric matrix that gives a protection to the cells against antibiotic agents, acid and temperature. The photodynamic therapy is a solution for kill a biofilm of S. aureus cells using blue light, curcumin as a photosensitizer and cellular oxygen. Different formulations and different ways to delivered the photosensitizer were tested. A curcumin film, curcumin dissolved in the surfactant tween 80, a nanoskin soaked with curcumin and curcumin dissolved at dmso and alcohol were the formulation tested at S. aureus and Escherichia coli biofilm. The time of the biofilm developed (1-7 days of developed biofilm) as the concentration, incubation time of the curcumin and the dose light were tested to optimize the experiments. Different type of illumination was tested, testing the irradiance and the time dose for kill the cells. An experimental designs were planned and the PDT was optimized.

S3-17. Internalization of photodithazine in c.albicans microbial wall for photodynamic therapy

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Photodynamic therapy is a technique used in microbial decontamination and treatment of infections consisting of the union of a photosensitizing agent, light at the appropriate length and oxygen to trigger the generation of cytotoxic agents. The interaction and action of the Photodithazine® molecule as photosensitizer (FS) in Candida albicans (CA) was characterized. Temporal and spectral internalization experiments of the PDZ in CA were performed by confocal fluorescence microscopy with excitation by 1 (1P) and 2 photons (2P), to determine the uptake of FS by CA. Demonstrating a high internalisation induced by the excitation light for FS. This induction can be performed in low doses of 1P and 2P light and favors a homogenous FS internalization. We suggest the proposition of new PDT protocols that inserts a low dose pre-illumination during the incubation process to improve the action of FS with the microbial wall. Experiment with excitation of light from LEDs (600 nm). In this study aimed at cell viability with continuous doses of light (single dose) and serial doses, the serial doses were applied so that the light exposure was quantified in 1J.cm-² with a dark interval of 2 minutes. The results demonstrated that the serial application promoted greater FS entry in the CA cell and showed earlier cell death compared to the

single dose of the same value. Further studies will be conducted to predict the behavior of this early PDZ internalization.

S3-18. Investigation of photodynamic inactivation and ultraviolet radiation for decontamination of transplantation organ grafts - animal model

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The need for organ transplantation is increased in the world in an excessive way, being the biggest challenge the huge line of people that need a transplant and the low number of organs available to be transplanted. Currently, one of the problems that prevent the transplant from being performed is that the organ of the donor is infected by pathogenic microorganisms. A Photodynamic Therapy (PDT) or Photodynamic Inactivation (PDI) is a promising modality of therapy for the inactivation of pathogenic micro-organisms. In this study we propose the evaluation of photodynamic inactivation and ultraviolet radiation as an alternative for the decontamination of organs for transplantation infected by pathogenic microorganisms, being a relevant potential for increasing the availability of organs of transplant networks.

S3-19. Treatment of Pharyngotonsillitis with Photodynamic Action

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Antimicrobial Photodynamic Therapy (APDT) is being increasingly used for treatment of acute infections. The cause of upper respiratory tract infections represent a large part of the diseases caused by drug-resistant microorganisms. Acute pharyngotonsillitis caused by bacteria represent many cases that are admitted to hospital emergency daily. Antibiotics are the first line treatment for bacterial pharyngotonsillitis. However, drug failure may occur by antibiotic therapy, which can cause recurrent pharyngonsilitis. Pharyngotonsillitis treatment has been studied by Optics and Photonics Research Center (CEPOF) for the last five years. The studies were focused on the following tests: determination of a formulation used in tonsils; development of lighting device for tonsils; development of clinical study phase I and II. The results have shown that under specified the PDT can be used for the treatment of pharyngotonsillitis. It was possible to observe the increase of incorporation of photosensitizer depending of formulation composition; the microbial resistance behavior in relation to successive PDT sessions; the safety of the technique in clinical phase I study, and clinical results, not yet completed, in phase II showed the PDT efficient against different types of pathogenic microorganisms in adults.

S3-20. An update on photodynamic therapy in the treatment and diagnosis of onychomycosis.

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The onychomycosis is one of the most prevalent nail nail disorders, caused by filamentous fungi and dermatophytic and non-dermatophytic yeasts. This infection is difficult to treat and, sometimes, isrefractory to conventional therapy, which consists of the administration of antifungal agents for long periods of time. Due to the increase of drug-resistant microbial strains and the high incidence of this infection, it is necessary the development of new technologies, such as photodynamic therapy (PDT). In recent years, PDT has been extensively studied in hopes of finding efficacious and suitable treatment modality for onychomycosis. It is a non-invasive therapy that utilizes light to activate a photosensitizing agent applied topically or systemically, which generates reactive oxygen species (ROS) that initiate the destruction of cells by necrosis or apoptosis. The purpose of this study is to implement PDT as a technique for the treatment of onychomycosis, using a Brazilian curcumin as photosensitizer (PDT Pharma, Brazil), incorporated in different formulations. The suggestion of a new diagnostic tools using thermographic and fluorescence imaging andcarry out a clinical study for the treatment of patients with onychomycosis using a specific device for this application are other aims of this study. In clinical trials, thermographic and fluorescence images have been shown to be helpful in the diagnosis of onychomycosis.

The clinical results, after the treatment of 50 patients with mild-to-moderate toenail onychomycosis, showed good response with differents number of sessions of PDT and dependence withthe formulation used. This clinical study is part of the ongoing research with methods of PCR diagnosis - Polymerase Chain Reaction, correlating the bacterial strain with the success of the treatment. The low cost, the simple operation with fast clinical results are important factors for the implementation of this technology in the treatment of onychomycosis.

S3-21. New formulations with curcumin for the treatment of onychomycosis by photodynamic therapy

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Onychomycosis is a disease of high incidence in the nail plate and responsible for approximately half of the cases of nail infections. Conventionally, dermatologists prescribe antibiotics and antifungals for long periods for its treatment. However the nail unit acts as a barrier to exogenous substances, its physiological features hampers drug penetration, turning the onychomycosis treatment a challenge. In light of these facts, we propose photodynamic therapy (PDT) as an alternative treatment of onychomycosis. PDT is a promising technique by which microorganisms are eliminated by a photosensitizing compound , light and oxygen. Curcumin is a natural product that can be obtained from the Curcuma longa rhizome and has become promising as as a photosensitizer. The aim of this research is study the curcumin as it is a natural in different formulations modifying the vehicle to improve photosensitizer delivery trough the nail plate, hence increasing therapy effectiveness. Was study four different formulations and analysed by spectroscopy raman, confocal, pH, and organoleptic properties, and the most stable formulation was determined. As well the samples were conditioned in stability chambers with controlled temperature and humidity and were analyzed at the recommended times. The gathered may be useful for the development of safer and more effective at low cost and low probability of side effects for onychomycosis treatments.

S3-22. Reduction of gene expression related to Candida albicans adaptation to oxidative stress after photodynamic therapy mediated by Photodithazine

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Candida albicans is considered a commensal microorganism and in situations of immunological imbalance, this fungus becomes an opportunistic pathogen. Photochemoterapy (aPDT) has been used for inactivation of these microorganisms and requires the application of a photosensitizing (PS), a light source that corresponds to the PS absorption band, and oxygen. The present paper evaluated the effect of Photodithazine (PDZ) on the genes' expression responsible for the fungal response to oxidative stress (CAP1, CAT1 and SOD1) in biofilms of C. albicans. Strains of C. albicans ATCC 90028 were submitted the formation of biofilm. After, 100 and 200 mg/L of PDZ was applied to the biofilm, which was first incubated 20 min at pre-irradiation and subsequently exposed to 37.5J/cm2 and 50J/cm2 LED light doses corresponding to 660nm PS concentration. In addition, the group received the light and PDZ (P+L+), the samples were treated only with PS (P+L) or light (P-L+). The control group (P-L-) did not receive any type of intervention. The biofilm was detached and subjected to extraction and purification of RNA, conversion to cDNA and performance of the qPCR assay. The samples were processed in the CFX96 (BioRad). PDZ-mediated aPDT reduced 99% expression of the SOD1 gene, regardless of the concentration or dose of light, when compared to the P-L-. In the CAP1 gene reduction was observed not only when compared to the P-L- group, but also in the P+L- group. The highest reduction in CAT1 expression was obtained with the application of aPDT with 200mg/L associated to 50J/cm2. The highest reduction in CAT1 expression was obtained with the application of aPDT with 200mg/L associated to 50J/cm2, also in comparison to

the control group. Analyzing the results obtained in the present study, it is suggested that the PDZ-mediated aPDT was able to reduce by more than 90% the expression of the genes. The use of PDZ e mediated by aPDT facilitates the action of reactive oxygen species on fungi.

S3-23. Evaluation of caries-removal ultrasonic device associated with photodynamic therapy in caries-like dentin induced by the biological model

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The treatment and prevention of caries disease should be studied in order to investigate novel therapies that contribute to its prevalence decrease. Thus, the aim of this study is to evaluate the effectiveness of the use of ultrasonic device associated with antimicrobial photodynamic therapy (TFA) in the removal and dentin decontamination and the application of bioactive materials to promote remineralization, as well as the influence of these therapies on adhesive strength. Artificial caries lesions in bovine dentin will be induced by the biological model using biofilm duo species. Then, dentin will be removed and decontaminated, and performed the following evaluations: cross-sectional microhardness, transverse microradiography (MT), confocal microscopy (MC) and FT-Raman. Afterward, bioactive agents will be applied to the remaining dentin and the remineralizing capacity and morphological changes of the dentin will be evaluated by means of MT, MC and FT-Raman. The lesions will be restored with composite resin and evaluated for the treatment effects on the adhesive strength and failure pattern, by means of micro-shear test, optical microscopy and scanning electron microscopy (SEM) images. After evaluation of normality and homoscedasticity, if they are met, the data will be submitted to analysis of Variance (ANOVA) and multiple comparisons. Otherwise, non-parametric analysis will be used.

S3-24. Monte Carlo evaluation of the light distribution within the thoracic cavity using extracorporeal radiation

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The purpose of this project is to study and understand the spatial distribution and penetration of extracorporeal infrared radiation into the thoracic cavity with multiple emitters using Monte Carlo simulations. The Monte Carlo method provides a flexible and rigorous solution to the radiative transport equation, an integrodifferential equation that describes the light-tissue interactions, taking into account factors like optical properties, characteristics of the light source, composition of the tissue, among others. This study uses an optimized method, Monte Carlo eXtreme (MCX), which has been widely used in recent years because it presents the results in a fairly short compilation time and with a certain ease of use. Under this context, the effect of a single source for the different layers of the thoracic cavity is analyzed first, then the following step is the analysis of multiple sources and the comparison: the fluence rate, the amount of absorbed and scattered photons, and the volumetric distribution of two wavelengths, 780 and 808 nm. This study is part of a larger project in our research group that aims to treat bacterial pneumonia with photodynamic inactivation using extracorporeal irradiation. It has advanced in experimental terms of the technique and inactivation of the bacteria, showing promising results in vitro and in vivo. This theoretical understanding the light-tissue mechanism intends to help our research group design and take the next steps required for the protocol to become a clinical treatment.

S3-25. Evaluation of photodynamic effects of naturally occurring photosensitizers on Aedes aegypti larvae

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Transmission of diseases such as dengue, Chikungunya, Zika and yellow fever have been a problem on national territory in recent years with high numbers of probable cases. These diseases are transmitted by mosquitoes of the genus Aedes and there are yet no vaccines or medicines available for dengue, Zika or Chikungunya. Vector control has been made mostly by chemical insecticides, which has led to the development of resistant populations. Thus, the search for other forms of control became necessary, and the use of products derived from plant sources represents an important alternative. Photodynamic Inactivation is based on the use of a photosensitizing molecule that is activated by light at a specific wavelength, forming reactive oxygen species that cause cellular damage and tissue destruction. In this study, curcumin, a molecule from the rhizomes of the Curcuma longa plant, will be used as a photosensitizer. Its photodynamic effects has been studied in the microbiological control and against larvae of the Aedes aegypti mosquito. Therefore, this study aims to analyze the effects of photodynamic action of curcumin on the development of Aedes aegypti under sublethal conditions.

S3-26. Study of curcumin photodegradation and photodynamic action in Aedes aegypti larvae

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Yellow fever, dengue, chikungunya, and zika are diseases of high incidence in subtropical and tropical countries. The control of the Aedes aegypti vector transmitting of these diseases is based on the use of conventional chemical insecticides. However, the excessive and continuous application of insecticides have induced the selection and proliferation of resistant mosquito. In this sense, the present study aimed to use curcumin as photosensitizer for photodynamic control (PDC) of Aedes aegypti larvae under sunlight irradiation as well as to evaluate its photostability and the photoproduct generated during its degradation. The PDC experiments were carried out submitting the 3rd instar stage larvae to curcumin at concentration of 4.6 mgL-1 under sunlight irradiation, in the natural environment, during 22 days. The photostability analysis was performed using a solar simulator with irradiance of 100 mWcm-2 (1 sun) to stimulate an accelerated degradation of the curcumin. Photodegradation was determined by monitoring the UV-Vis absorption and liquid chromatography with mass spectrometer (LC-MS). The results of the PDC showed that curcumin induced a larval mortality of 92 %. The degradation assay revealed a decrease of 79, 67 % in the absorption intensity at 430 nm after 120 min of irradiation. In addition, there was observed a light absorption increase in the UV region (220 and 280 nm), associated to the generation of degradation products. Although preliminary, the LC-MS results demonstrate that during the photodegradation process the formation of 13 intermediates of m/z = 172, 194, 200, 212, 226, 242, 278, 290, 368, 370, 402, 418 and 434.

Keywords: Photodegradation; Aedes aegypti; photodynamic control; irradiation

S3-27. Eosin methylene blue to photodynamic control of the Aedes aegypti population

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Aedes aegypti (Ae. aegypti) is a competent vector for transmitting important viral diseases such as yellow fever, dengue, chikungunya, and zika. Strategies have been applied to avoid the Ae. aegypti proliferation by using environmental, biological, and chemical approaches. However, other methods for the effective controlling of the insect vector population is still needed. Photodynamic control is an alternative way to handle the vector population, being a physical approach based on the larval phototoxicity induced by a photosensitizer. The present

study tested the eosin-methylene blue (EMB) as a new photosensitizer for photodynamic control of the Ae. aegypti larval population. The photodynamic assays were performed submitting third-instar larvae to two EMB concentrations (0.0, and 100.0 mg.L-1) under different light doses (96.3 and 165.06 J.cm-2). White-light radiation or wavelengths (450, 525, or 625 nm) from RGB LEDs were tested as excitation source. The results showed that the EMB is rapidly internalized in the larvae and it is phototoxic for Ae. Aegypti, regardless of the radiation source.
Keywords: Aedes aegypti; larval control; photodynamic process; eosin-methylene blue

S3-28. Cationic imidazolyl phthalocyanines : structure-activity studies for antimicrobial therapy

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Recently, no new antibiotics have been discovered, which would delay the onset of infections by multi-resistant bacteria. This enormous urgency to develop new molecules has trigged an increasing interest in antimicrobial photodynamic therapy (aPDT). Our long experience on the synthesis of photosensitizers for inactivation of tumour cells led us to direct our studies toward the synthesis of new cationic phthalocyanines bearing imidazole groups and strong absorption in the near infra-red region. Herein we present and discuss our developments on the synthesis of a family of cationic phthalocyanines with varying structural features such as size of the cationizing alkylic chain, degree of cationization and central coordinating metal, which were obtained in good yield (42-71%). The influence of these structural modifications in their spectroscopic and photophysical properties, reactive oxygen species (ROS) generation, as well as biological performance was assessed. All compounds showed good photophysical and photochemical parameters, namely high absorption coefficients and singlet oxygen production quantum yields. The antimicrobial activity of these new entities in combination with white light was tested against gram-positive, gram-negative bacteria and fungi. Remarkable differences were found among all cationic phthalocyanines as some were highly active in killing gram-negative species (E. coli, P. aeruginosa) and C. albicans in the nM and uM scale while leaving mammalian cells relatively unharmed, which makes them promising leads for treatment of localized infections. We found a unique structure-activity relationship for this family of cationic tetra-imidazolyl phthalocyanines in the sense that it does not follow what was previously established by other authors, which may give new insights on the rational design of photosensitizers for use in aPDT. Ref: R. T. Aroso, M. J.F. Calvete, B. Pucelik, G. Dubin, J. M. Dabrowski, L. G. Arnaut, M. M. Pereira. (Submitted)

S3-29. Bacterial Cellulose-based Cell Culture Platform for Biomedical Application

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Cell culture platform is a versatile device that can be applied in the medical and biomedical field to investigate cellular responses to drug administration (drug screening), as disease models for the development of suitable treatments, in cell cryopreservation, and also while scaffolds in tissue engineering. Bacterial cellulose appears as an interesting support material, as it presents high biocompatibility, it exhibits three-dimensional architecture similar to the extracellular matrix through the presence of entangled nanofibrils and it shows more adequate physical and mechanical properties compared to vegetable cellulose. Due to the presence of hydroxyl groups on the cellulose surface, a wide variety of derivatives can be prepared by modifying its original functional group. In this sense, the present study aims to functionalize the surface of bacterial cellulose by chemical processes followed by the immobilization of growth factor, in order to obtain optimized cell cultures platforms.

S3-30. Low-Cost Visible Light Photocatalyst from Bimetallic Doped Clay

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Almost two billion people today drink water mostly contaminated with feces, thus putting them at risk of contracting various waterborne diseases (WHO, 2017). Nanocomposite materials were developed from kaolinite clay, agro-waste and ZnCl2 for efficient water disinfection. Fixed-bed mode under gravity was used for disinfection process while steam regeneration method was employed to evaluate the re-usability of the most efficient composite material. We show for the first time that, doping the hybrid clay material with a binary mixture of transition metal salts (ZnCl2 and CuCl2) extends the efficiency of our prepared material. The enhanced nanocomposite materials were evaluated via the removal of both non-resistant and multidrug resistant (MDR) Escherichia coli (E. coli). Nanocomposites prepared with bimetallic salts of Cu/Zn provided the best disinfection efficiency against E. coli with a breakthrough time of 36 h for the removal of 2.32 x 107 cfu/mL whereas single metal salt nanocomposites exhibited a breakthrough time of 30 h and 25 h respectively for the same amount of E. coli. X-ray diffraction, Scanning electron microscope, Raman and Fourier transformed infrared analysis indicates the successful doping of the metal salts into the hybrid clay nanocomposites with new phases observed. UV-vis diffuse reflectance and Photoluminescence spectroscopy revealed that both Cu-doped and Cu/Zn-doped nanocomposite materials were visible-light active with the generation of reactive oxygen species while Fluorescence spectroscopy was further used to confirm that singlet oxygen was responsible for the inactivation of the test organism. Moreover, no bacteria regrowth was observed after 4-days. This long hydraulic times and the good regeneration capacity of the composite materials especially Cu/Zn-doped nanocomposites, makes them a potential functional material for development of simple point-of-use water treatment systems for water disinfection application.

S3-31. Dissolving microneedles with aminolevulinic acid for PDT – a pilot study in human skin

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Photodynamic Therapy (PDT) is a therapeutic modality that has been applied mainly to skin cancer treatment. For topical PDT, the photosensitizer (PS) precursors are usually applied in a cream form. Aminolevulinic Acid (ALA) and its derivatives are the most common photosensitizer's precursors used, allowing the Protoporphyrin IX (PpIX) accumulation. One of the challenges in topical PDT for skin cancer is to increase the cream permeation to improve the success rate and treat deeper lesions. Microneedles (MNs) are minimally invasive systems already used for intradermal vaccination, delivery of systemic drugs such as antibiotics or hormones. In this study, we prepared dissolving MNs using 5% ALA concentration and 20% of Gantrez AN-139 polymer. The patch has 361 pyramidal microneedles with 500 μm high. The tests of mechanic strength, dissolution, and stability of MNs were encouraging to perform this study. Three healthy volunteers received the MNs patches on the forearm, and optical coherence tomography (OCT) images were collected showing the induced microholes in the skin after the MN removal. We compared the superficial PpIX formation of the dissolving MNs and the standard cream at 20% concentration by a fluorescence spectroscopy system. The study showed that even in lower concentration, the MNs were able to produce a similar amount of PpIX compared to the cream in the same incubation time. The results were encouraging to perform an animal tumor model to support the understanding of the PDT.

S3-32. Long-term effectiveness and HPV clearance of low and high-grade cervical lesions treated with photodynamic therapy

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Cervical cancer is the second leading cause of female cancer in Brazil, with about 16,370 new cases estimated for each year of the biennium of 2018-2019 [1]. Persistent infection with Human papillomavirus (HPV) has been identified as the major cause of the Cervical Intraepithelial Neoplasia (CIN), a precursor of cervical cancer. The classification of CIN is based on the cellular features to discriminate dysplasia levels, being CIN 1 as mild dysplasia and CIN 2/3 as moderate or severe dysplasia. [2]. Cervical cancer can be prevented with a early CIN diagnosis and treatment [3]. After two years 15% of untreated CIN 1 could progress to CIN 2/3 and these high-grade squamous intraepithelial lesions (HSIL) should be immediately treated with excision. The present study reports the results of a controlled randomized clinical trial for CIN 1, 2/3 treatment. The follow up was performed at 30, 60, 90, 180 days, and at 2 years after PDT with colposcopy, Pap test, and biopsy in HSIL cases. CIN 1 (n = 70), CIN 2 (n = 10), CIN 3 (n = 08) and placebo group (n = 15) were treated with different protocols. The total rate of complete response was 75% for CIN 1 (2 years after PDT), 21.43% for placebo group, 67% for CIN 2 (1 year after PDT) and 62.5% for CIN 3 (60 days after PDT). The results of hybrid capture are showing a significant decrease (70-80%) in viral load. To improve the results for HSIL treatments, was coupled a laser to illuminate the endocervix.

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S3-33. Photodynamic inactivation of planktonic and biofilm growing bacteria by imidazolyl cationic porphyrins

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The worldwide emergence of multidrug-resistant (MDR) bacteria are considered by the World Health Organization (WHO) one of the main causes of mortality by infectious diseases. It has been estimated that more than 80 % of all microbial infections are caused by formation of bacteria biofilms.[1] According to WHO recommendations, an urgent investment in R&D is essential for the development of new antibacterial entities with alternative mechanisms of action, to avoid that around 10 million people will die annually worldwide by 2050.[2,3] Antimicrobial photodynamic therapy is one of the methodologies that has received significant attention, for not being associated with the development of microorganism resistance after treatment.[4] The present work intends to overcome the challenges of MDR bacteria by the development of new photosensitizers based on cationic imidazolyl moieties with different molecular weights. Their antimicrobial activity was tested towards a panel of pathogenic microorganisms: Gram-positive (Staphylococcus aureus and Enterococcus faecalis), Gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa) and S.aureus biofilms. Total inactivation was found for concentrations as low as 100 nM in planktonic bacteria with irradiation at 415 nm (LED, 1.36 J/cm2). On the other hand, in S.aureus biofilm, we observed the molecular weight effect and an irradiation with 5 J/cm² in the presence of 5.2 nM of the smaller photosensitizer showed an impressive destruction of the biofilm (~99,43 %).

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S3-34. Energy fluence rate as a key parameter for modulating the efficacy of methylene blue photodynamic therapy against pancreatic cancer cells

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Pancreatic ductal adenocarcinoma is the fourth cause of cancer related death in the western world. Resective surgery, alone or combined with chemotherapy, remains the only effective therapeutic approach for treating this disease. However, tumor's recurrence is observed less than one year after surgical intervention. Photodynamic therapy (PDT) appears as a promising alternative treatment for fighting cancer. Nevertheless, there are few systematical studies investigating not only the influence of different parameters related with PDT efficacy of killing tumor cells but also the molecular mechanisms triggered by different protocols. In this study, we set out to evaluate the influence of the energy fluence rate in the efficacy of PDT using methylene blue (MB) as a photosensitizer (MB-PDT) on human PDAC cells (AsPC-1, BxPC-3, MIAPaCa-2 and Panc-1). The strategy used was to vary the irradiation time while maintaining the energy fluence (4.5 J/cm2). We observed that MB-PDT with 6 min of irradiation was able to induce significant death in all cell lineages. Nevertheless two different response profiles were observed. Indeed, BxPC-3 and AsPC-1 were significantly more susceptible to MB-PDT than MIAPaCa-2 and Panc-1 cells. This diminished susceptibility was abrogated by increasing the irradiation time to 16min. We also observed a significant increase in intracellular oxidative stress as well as a decrease in GSH levels. Additionally, we have shown that the fact that Panc-1 and MIAPaCa-2 cells have limitations for triggering necropotosis, a regulated cell death pathway already involved in PDT-induced cell death when using shorter irradiation times, did not inhibit the cytotoxic properties of the therapy when lower energy fluence rates were used. Collectively, our results have shown not only that irradiation time is a key parameter capable of modulating MB-PDT efficacy but also that it is decisive for orchestrating the cell death pathways followed by tumor cells upon MB-PDT.

S3-35. Hybrid nanomaterials for improving the phototherapeutic treatment against cancer

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Hybrid nanomaterials have gained popularity with their ability to combine a multitude of components to allow for systemic tuning of the properties of the resultant nanoparticles. Herein, we designed, fabricated and examined two distinct novel hybrid nanomaterials (HNMs) for improved PDT against cancer treatment. The first platform is polysilsesquioxane nanoparticles fabricated using protoporphyrin IX (PpIX-PSilQ) silane derivative as the building block forming the PSilQ network loaded with curcumin as the chemotherapeutic agent. Spherical nanoparticles of 40nm in diameter and high loading of PpIX (24.4±2.5%wt) and curcumin (7.6± .5%wt) were obtained. The combined phototherapeutic effect of PpIX-PSilQ(cur) NPs was evaluated against multiple cancer cell lines. A synergistic therapeutic effect was observed in vitro for the combination of PpIX and curcumin implicating great promise in combinatorial treatment against cancer using PDT. The second platform studied is nuclear targeted polymeric micelles (PM) encapsulating verteporfin (VP) and CeF3 nanoparticles as the scintillation nanocrystals (ntPM-VP-NC). The hypothesis is that the deep penetrating X-rays will activate the NCs which will trigger subsequent FRET between the co-localized NCs and PSs resulting in high PSs activation efficiency and at the same time overcome limited tissue penetration in conventional PDT. The ntPM-VP-NC with diameter of ~100nm was fabricated with >500 NCs per PM. Preliminary data showed enhanced phototoxicity for ntPM-VP from the nuclear localization of the PSs in comparison to the non-targeted analogue (non-ntPM-VP) against BxPC3. Singlet Oxygen generation and DNA damage evaluation presented with cumulative evidence in support of our hypothesis. Overall, these HNMs show tremendous potential of becoming new therapeutic modalities with the possibility of improving the current photodynamic therapy to treat cancer.

S3-36. Optical strategies to improve PDT response on cutaneous, conjunctival and metastatic melanoma

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Melanoma originates from the melanocytes, which are pigmented cells present throughout the body. The primary therapeutic approach is surgery or enucleation. Photodynamic therapy (PDT) is based on the interaction of light and photosensitizer, leading the cell to death with increasing evidence of an immune component. Since melanoma is often highly pigmented, it does not respond well to conventional PDT due to the high absorption of light that makes volumetric eradication impossible. We hypothesize that PDT combined with an optical clearing agent (OCA), 2-photon excitation PDT (TPE-PDT), and X-ray activated PDT (XPDT) can overcome this limitation in cutaneous, ocular melanoma, and metastatic melanoma, respectively. OCAs are hyperosmotic agents that reversibly dehydrate tissue that reduces tissue scattering and improves light penetration. We have demonstrated in vivo that OCAs can double the depth of light penetration into pigmented melanoma, allowing high-resolution imaging of the microvascular network. Subsequently, we could eradicate cutaneous tumor with a single OCA+PDT session. TPE-PDT relies on the use of ultrashort near-infrared laser pulses to excite the photosensitizer, rather than low-intensity continuous light used in standard clinical PDT. In vitro studies have shown that melanotic cells are markedly more susceptible to TPE-PDT than amelanotic cells, which raises fundamental mechanistic questions. Also, in a conjunctival melanoma model, we could eradicate tumor, assessed by the short-term response. X-ray activated PDT uses of X-rays to excite directly or indirectly the photosensitizer. In our study, we are using a scintillator that converts the X-ray irradiation into the light to excite the photosensitizer. These studies are ongoing, and I will present the first results in cells. These investigations aim to develop optical strategies to improve PDT efficiency in melanoma models, still not successfully addressed by photonics techniques.

S3-37. Analyze of photobleaching of PpIX using different light sources and theoretical estimation of singlet oxygen dose by explicit dosimetry in PDT

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The protoporphyrin IX (PpIX) is an important photosensitizer molecule to photodynamic therapy (PDT). By molecular symmetry, the PpIX has one Soret band (408 nm) and four Q-bands (506, 540, 575 and 630 nm) of absorption. We investigated the change on Soret, Q-bands and photoproduct formation as function of time and volumetric dose of absorbed photons to different light sources. The light sources are two LED with maximum value in 407 (violet), 72 mW/cm^2 and 639 nm (red), 175 mW/cm^2. Three experimental with different photoirradiation configurations were made (a) violet, (b) red and (c) violet and red. The volumetric dose of absorbed photons is 1x10^19 Phot./cm^3. The irradiation time is calculated by division between volumetric dose of absorbed photons and overleap between spectral emissions of light source and spectral absorption coefficient of PpIX. The initial concentration of PpIX is 5 μM in DMSO. To all absorption bands considering the same number of absorbed photons, the results show different values of decay time constant and same decrease in the amplitude PpIX absorbance. The rate of formation of photoproduct (band 653 nm) absorbance amplitude is different. To estimate de singlet oxygen dose, a mathematical model was developed to the type II mechanism of PDT together with explicit dosimetry, i.e. considering the local fluence rate and concentrations of PpIX and oxygen. To this mathematical model the initial condition is the spectral irradiance to each light source, the absorption coefficient spectrum to PpIX and the concentration in μM of PpIX and oxygen

S3-38. How does photosensitization alter membrane microdomains?

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Photodynamic Therapy (PDT) has been the focus of research and development to facilitate medical field application. Despite advantages of PDT for a variety of applications, it has not achieved an equally prominent position in clinical practice. A critical aspect during PDT treatments is the PDT efficacy and the determination of accurate treatment protocols. One of the main strategy used to increase PDT efficiency by several authors is based on photosensitizers (PS) that interacts strongly with membranes, since lipid membrane are important target of photosensitized oxidations. In PDT, it is well known that the role of membrane interaction with photosensitizers is crucial to the extension of photo-induced damage and consequently to the efficiency of cell death. Although the general consequences of lipid oxidation during PDT is well known, the impact that photosensitization may trigger on rafts membrane microdomains are still poorly understood. Therefore, we investigated the phase separation behavior of two oxidized lipids bearing different shapes, POPCOOH as the cylindrical and PazePC the conical one, in ternary mixtures-containing membranes. The results show in an unprecedented way that POPCOOH promotes formation of micro-sized lipid rafts membranes while PazePC disarrange the lipid micro-domains. Our findings provide insights about the behavior of lipid rafts, mediated by oxidative stress, contributing to better comprehension of its at molecular level.

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S3-39.Multi-session PDT in 3D cell culture of breast cancer

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Photodynamic therapy (PDT) has been investigated in clinical studies as a treatment method for some types of cancer. Some protocols use more than one PDT session and there are cases in which a remaining tumor is present. In vitro studies are crucial to guide the treatments and to understand the outcome, in particular death mechanisms and the possibilities of cell developing resistance and getting more aggressive. For these investigations, tridimensional (3D) cell cultures models stand out compared to monolayer culture since they can provide more similarities with the tumor natural microenvironment such as cell-cell and cell-extracellular matrix interaction. The purpose of this study is to investigate low-dose multi-session PDT in 3D cultures of breast cancer cells grown by the magnetic levitation method. Three PDT sessions were performed 48 h apart with Photodithazine at a concentration of 50 μg/mL and LED irradiation at 660 nm. Macroscopic damages of different intensities were observed in the spheroids after each session. The live/dead assay performed 24 h after the last PDT session showed a higher live to dead cells rate compared to control, even though the spheroid morphology presented small changes. Alterations in proteins and other molecules in the cell-cell interface and in the cell membrane are being investigated with Confocal Raman Microscopy. The combination of these results can provide information about death mechanisms of PDT in 3D tumors. The follow up of spheroids for some days after the PDT sessions can be used to investigate the effects of this therapy in remaining tumors.

S3-40. Study of vascular and photosensitizing effects of curcumin using chorioallantoic membrane model

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Photodynamic Therapy (PDT) is a treatment that requires light, a photosensitizing agent and molecular oxygen. The photosensitizer is activated by light and interacts with the oxygen present in the cellular microenvironment. The molecular oxygen is transformed into singlet oxygen, which is highly reactive and responsible for the cell death. Therefore, PS is an important element for the therapy happens, including its concentration. Curcumin is a natural photosensitizer and it has been demonstrated its anti-inflammatory and anti-oxidant effects that inhibit several signal transduction pathways. PDT vascular effects of curcumin at concentrations varying from 0.1 to 10

mM/cm² and topical administration were investigated in a chick Chorioallantoic Membrane (CAM) model. The irradiation was performed at 450 nm, irradiance of 50mW/cm² during 10 minutes, delivering a total fluence of 30 J/cm². The vascular effect was followed after curcumin application, with images obtained each 30 min in the first 3 hours, 12h and 24h. Those images were analyzed qualitatively and quantitatively with a MatLAB®. Curcumin was expected to exhibit a vascular effect due to its angio-inhibitory effect. Using Curcumin as a photosensitizer, PDT induced a higher and faster vascular effect when compared to the photosensitizer alone.

S3-41. Evaluation Of Photodynamic Therapy Associated With Optical Clarification In The Treatment Of Cutaneous Melanoma

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Melanoma is a major public health problem because, despite the low incidence, it presents high rates of morbidity and mortality when diagnosed in more advanced stages. Thus, there is a need for the development of therapeutic options. Photodynamic therapy is a technique based on the use of a compound called a photosensitizer, of light of a specific wavelength for the excitation of the photosensitivity and of the oxygen present in the tumor tissue. The photodynamic reaction for induction of cell death occurs mainly by the production of singlet oxygen, a highly reactive and oxidative species. In the case of cutaneous melanoma, due to the high concentration of melanin, one of the main biological absorbers, photodynamic therapy presents a poor response due to the great limitation of light penetration into the tumor. Optical clearing agents have been used to make the tissues transparent, especially in biological samples for confocal microscopy. Our strategy is to use photodynamic therapy associated with melanoma pretreatment with clearing agents to optimize tumor illumination. In this study, we will evaluate the photodynamic response associated with optic clearing for the treatment of cutaneous melanoma.

S3-42. Influence of external factors in dose threshold distribution in photodynamic therapy and its relation to therapy resistance

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The Photodynamic Therapy (PDT) research field is on constant growth and has become a possible choice to treat non-melanoma skin cancer and cervical intraepithelial neoplasia. Although PDT has been associated to low risk of cell resistance so far, it is known that cells could overcome PDT due to photosensitizers (PS) and/or light dose variations, that can naturally occur according to tissue optical properties. In addition to that, metabolic diseases have the potential to interfere with drug-administrated treatments, but it is not known if disorders related to high concentration of glucose or low-density lipoprotein (LDL) could affect PS delivery and PDT response. So far, establishing the PS concentration or light dose to evaluate how tumors respond to treatment relies on researcher's choice. The purpose of this study was to evaluate if external factors as glucose or LDL could interfere with PDT in cell culture and how would cells respond to different PS concentrations and light doses. For this study, the photosensitizer Photogem was used to evaluate HepaRG cells response to PDT when adding a glucose or LDL concentration range. Results show that glucose concentrations as high as 30 mg/mL do not affect PDT efficacy, while LDL seem to modulate the response differently in the presence or absence of PS. When no PS was used, LDL seemed to contribute to cell death of the illuminated samples, but when the PS was added, LDL protected cells from PDT and cells responded to treatment only after higher doses of light when compared to control group. These results show that lipid and cholesterol disorders have the potential to interfere with PDT treatment, decreasing PDT efficacy in lower doses of light, which could result in more sessions of treatment to a successful outcome. Also, dose threshold analyzes could contribute to establish new clinical protocols according to the patient's condition when submitted to PDT treatment.

S3-43. PDT Dosimetry Using 3d Cell Culture Assembled By Magnetic Levitation

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In recent decades a substantial effort has been devoted to the study of dosimetry, effectiveness and safety of Photodynamic therapy in the treatment of many types of cancer. Several applications of PDT have proven to be effective, for example, early non-melanoma skin cancer and premalignant lesions. Still, the study of the effectiveness of PDT in the treatment of bulky tumors remains unclosed. Because PDT effect depends on the light penetration into the tissue, the damage necessary to eliminate a bulky tumor should be fractionated in multisession applications in order to eliminate layer by layer the tumor. Understanding the multisession PDT procedure demands an effort in the understanding consequences of tumor dynamics and complexity after PDT damage after every application. New models for PDT multisession dosimetry has been studied and PDT dosimetry in vitro has followed the biotechnology trend to use 3D cell cultures that presents certain level of similarity with native cancer tissue. The 3D cell culture model stands between the traditional monolayer cell culture and the animal model, and its use can reduce the number of animals used in dosimetry of PDT. The magnetic levitation method is based on the interaction of cell membranes with nanoparticles containing iron oxide and magnetic fields generated by specific magnetos. After the 3D cell cultures assembling the first PDT application was performed using the photosensitizer Photogem.

S3-44. Comparison between Photodynamic Therapy and trichloroacetic acid for Condylomas acuminate treatment

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It is estimated that up to 75% of the sexually active population is infected with this human papillomavirus virus (HPV). Condylomas acuminate are benign lesions caused by this virus. This study aims to compare photodynamic therapy (PDT) and administration of 70% trichloroacetic acid (TAA) in the condylomas. Each patient is treated weekly until total removal of the lesions, with one of treatment options. In the patients treated with PDT, a Brazilian 20% methyl aminulevulinate cream were applied in the region and incubated by 3 hours and the lesions were illuminated with the CERCa®. This Brazilian system is composed of LEDs emitting at 630 nm which was initially designed for the treatment of cervical intraepithelial neoplasia (CIN) and this study showed the need to develop new devices for illumination of the anus-genital region. During the illumination, a total dose of 150 J/cm2 is delivered over 21 minutes. 22 patients have participated of this clinical study, totaling 15 patients treated with TAA and 8 with PDT, with a complete treatment for 33% using TAA and 75% for PDT. Besides the results of complete response, it is important to highlight that 2 patients showed recurrence for TAA and 4 patients discontinued the treatment with TAA, while, for PDT, there were no cases both recurrence and withdrawal. Therefore, besides the preliminary results obtained with this project show that PDT has been more effective than TAA, it was possible to plan and develop new equipment to ensure greater range of light.

S3-45. Aminolevulinic Acid combined with thermogenic and/or vasodilator substances to optimize production of Protoporphyrin IX

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One of the limiting factors of photodynamic therapy is cutaneous permeation of a photosensitizer or precursor. Studies report that there is a strong relationship between temperature and porphyrin synthesis in biological tissue. The use of thermogenic and/or vasodilator substances may favour both ALA/methyl-ALA permeation and protoporphyrin IX (PpIX) production in the tissue. In this study, menthol, methyl nicotinate and ginger extract were incorporated into either the ALA or methyl-ALA cream to investigate the PpIX production in rat skin. Fluorescence spectra were collected to quantify the PpIX present in tissues. The methyl nicotinate was the one with the highest optimization effect of PpIX production after three hours of incubation of the cream. Its association with methyl-ALA

caused the production to be about 50% higher than that observed for methyl-ALA alone and about 67% higher than when associated with ALA. These results are promising as a possible strategy for decreasing the incubation time of the precursor cream in various clinical protocols and increasing the photosensitizer production in lesions.

S3-46. Development of a PDT system for treatment and monitoring of skin lesions via fluorescence images of PpIX around 700 nm region

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The high variation in the optical coefficients and the physiological properties of skin lesions produce a high variation in the PDT outcomes. Without the treatment monitoring, this could lead to unforeseen incomplete responses or non-responders, and finally to a lack of adoption around the medical field for skin lesion treatment. We developed a portable system to both treat and monitor ALA-based PDT in real-time during the therapy via fluorescence images of PpIX around 700 nm. Normally, when the PpIX fluorescence technique is chosen for PDT monitoring or dosimetry, the 405 nm is used to excite its fluorescence in the Soret band (the highest absorbance peak), producing a strong emission around 633 nm. To avoid the shallow penetration of violet range, due to the high absorption and scattering coefficients for skin molecules, the system excites PpIX with the treatment light itself, around 633 nm, and collects its fluorescence around 700 nm via camera in real-time during treatment.

S3-47. Raman Microspectroscopy Analysis Of The Response Of 3D Tumors After Photodynamic Therapy

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Cancer is responsible for 12% of worldwide deaths. The main treatments for cancer currently are: surgery, chemotherapy, radiotherapy and hormonotherapy. However, all these treatments have many side effects, damaging the patient's well-being, therefore development of a new, more effective methods of treatment with less side effects is needed. Photodynamic therapy (PDT) is a technique that associates light, molecular oxygen and photosensitizing agents and generates a cytotoxic effect on the target cell. The photosensitive compounds are excited by light at a suitable wavelength and react with molecular oxygen to form singlet oxygen that will induce cell death. Because of its selectivity, photodynamic therapy is a technique that, in clinical practice, has few or no side effects. The objective of this project is to evaluate the effects of photodynamic therapy in three-dimensional (3D) cultures of tumor cells using the Raman microspectroscopy. The confocal Raman microscopy is one of the most suitable methods for discovery of molecular mechanisms of the metabolic processes, and it is possible to study live cell samples without the use of markers. The three-dimensional cell cultures were used as a tumor model in vitro and the treatment protocol with photodynamic therapy was proposed not to cause complete tumor death, allowing the investigation of a remnant tumor formation. The Photodithazine (PDZ) was used as a photosensitizer. To analyze the 3D tumor the confocal Raman microscope Witec Alpha 300 RAS was used. The tumor analysis was done before and after the PDT. Measurements were taken to monitor the changes in the tumors during several days following the PDT treatment. Differences between the spectra obtained before and after PDT were identified and the profiles of these spectra are being evaluated to identify the molecules contributing to the signal and finally to describe the mechanism of PDT performance in 3D tumors.

S3-48. Biological signaling induced by photodynamic therapy in cholangiocarcinoma cells

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Photodynamic therapy (PDT) is a promising method for the treatment of non-resectable cholangiocarcinoma that has shown to be able to prolong of survival. However, while photodynamic therapy for cholangiocarcinoma patients performs better than palliative chemotherapy, it remains a non-curative intervention. One of the primary obstacles in its application as anti-cancer treatment is fluence attenuation through the tissue, leading suboptimal irradiation and ROS generation and activation of survival pathways. We hypothesized that tumor cells are also able to confer survival to other cells through paracrine signaling, possibly via extracellular vesicles. We investigated the paracrine signaling effects induced by photodynamic therapy in vitro, using conditioned medium generated with extrahepatic cholangiocarcinoma cells treated at both lethal and sublethal cumulative energy doses, on bystander cells. We found that at lethal cumulative energy doses, conditioned medium has cytotoxic properties, while this is reduced or absent at sublethal doses. By removing components in the conditioned medium through filtration or centrifugation, the cytotoxic effect could be reduced or even eliminated. Accordingly different factors are present in the conditioned medium that influence the bystander effect. It is concluded that PDT can induce bystander cytotoxicity. Multiple components in the cell culture supernatant are responsible for this cytotoxic effect.

S3-49. Fundamental Aspects Of Photodynamic Therapy Associated With Ultrasound For The Treatment Of Non-Melanoma Skin Cancer

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Photodynamic therapy (PDT) is a technique currently used as a first-line treatment for non-melanoma skin cancer. It involves three main components: a photosensitizing agent, a visible light source and molecular oxygen, which together cause cancer cells-death [1,4]. However, one limitation of PDT is that the visible light does not penetrate in deep lesions. Ultrasound allows sensitizer activation at greater depth due to its excellent penetration ability in biological tissue. Furthermore, it induces cell- damage through mechanical forces and chemical toxins according to the ultrasound frequency and intensity [2,3]. The current investigation involves three major phases (in vitro, in vivo and clinical) to evaluate the effectiveness of the association of photodynamic therapy with the ultrasound for the treatment of non-melanoma skin cancer. In this presentation, the results and analysis of the first phase are presented. To understand the effects of the direct interaction between excitation sources (i.e., ultrasound and red light) and the photosensitizer, 10 mL of solution composed by PpIX(5μM) and DMSO was irradiated simultaneously by red light (630 nm,30 mW/(cm)^2) and ultrasound (0.5-1.5 W/(cm)^2 ,duty cycle=50%). The absorption spectrum of PpIX after irradiation shows that the degradation of PpIX, due on the generation of ROS and hydroxyl radicals, is faster than irradiated by each source separately, improving the effectiveness of PDT. These results suggest that under the established parameters the treatment can be carried out in less time of exposure and with greater scope.

S3-50. Economic impact of photodynamic therapy in the Brazilian public health system for skin diseases treatment

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Non melanoma skin cancer (NMSC) can be seen as a multifaceted problem, considered primarily as a public health problem whose impact on society considers the morbidity and cost aspects of the treatment. It is a social problem, affecting all those who depend exclusively on the Brazilian public health system and need to wait months to receive any type of treatment. From the economic point of view, to treat all patients diagnosed with NMSC, it is necessary a big investment. Finally, the problem is logistical, since the territorial extension of Brazil and its population distribution do not enable the adequate care in all the places, which requires reallocation of patients from small cities to reference centers. Based on these facts, PDT for small skin lesions may be one of the best solutions from an economic point of view. Being a treatment that is easy for the training of professionals and enables to be performed in an ambulatory environment, minimizing post-treatment effects, this study shows that the cost of implementing the procedure on a large scale is extremely adequate for the national public health service. Using a strategy involving companies, national bank and medical partners, equipment, medication and protocols were tested in a multicenter study. With results collected over 5 years from a national program to implement PDT for non melanoma skin cancer over the Brazilian territory, we could reach a great economic evaluation of advances concerning the use of PDT for skin cancer.

S3-51. Morphological And Genotoxic Analysis Of PDT With Fotoenticine In The Treatment Of Gliossarcoma

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Gliosarcoma is a malignant neoplasm of the central nervous system. Despite advances in conventional treatment techniques, the prognosis of the patient remains unfavorable. PDT is a therapeutic modality consisting of the combined action of a photosensitizer (FS), light at wavelength appropriate to the absorption of this compound and molecular oxygen, generating reactive oxygen species that lead the tumor cells to death. The objective of this work was to evaluate the cellular viability of 9L / LacZ gliosarcoma cells, submitted to PDT with chlorine e6, to treat this type of malignant neoplasia, observing its action in vitro. FS Fotoenticine® was used at concentrations of 200 μg / ml to 6.25 μg / ml, in serial 1: 2 dilution, the light source was a LED-based device (Biopdi / Irrad-Led 660) in the region of 660 nm. In order to analyze the FS localization, the Rhodamine 123 (Rho 123) labeling was performed under a Confocal microscope, demonstrating the overlap of the drug with the mitochondria. The cytotoxic action was analyzed by mitochondrial activity (MTT), where a reduction of 93% in mitochondrial activity was obtained in the highest concentration after irradiation and 86% in the lowest concentration, demonstrating the potential of this FS for therapy. To identify the action of FS within the tumor cells, the lysosomes were labeled by Acridine Orange (LA) under fluorescence microscopy, and it was possible to observe the FS overlap on the LA marking, indicating the presence of FS in the lysosomes. New studies have shown that FSs that have a greater affinity with the mitochondria and the lysosomes, tend to be more effective in the death of the tumor cell. The cytotoxic test demonstrated a reduction in mitochondrial activity after treatment, leading to the hypothesis that interactions with cytoplasmic organelles contributed to cell death. Thus, Fotoenticine was shown to be promising in PDT for the gliosarcoma line, in the parameters used.

S3-52. Comparison of the application Aminolevulinic Acid and Photodithazine in Photodynamic Therapy in gliosarcoma cell line

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Photodynamic Therapy (PDT) has been increasingly studied for application in brain tumors, improving the prognosis of affected patients. PDT is based on the interaction of PS, light and molecular oxygen, for the generation of reactive oxygen species, which causes the tumor cell death. Aiming to improve the therapy, this work evaluates the cytotoxic action of PDT with the prodrug Aminolevulinic Acid (ALA) and a Chlorine, Photodithazine (PDZ), in gliosarcoma cells (9L/lacZ), maintained in DMEM medium supplemented with 10% of Bovine Fetal Serum and 1% Antibiotic. Initially, a Confocal microscopy analysis was performed to analyze the best incubation time for ALA, at a concentration of 125 µg/ml, for 1h, 2h, 4h, 6h and 8h. The times with the highest signal of the PpIX were the times of 4h and 6h. For the PDZ, the internalization was observed with 1h of incubation at a concentration of 200 µg/ml and it was possible to observe the presence of FS throughout the cytoplasmic area, including the perinuclear region. For cell viability, was performed Trypan tests for ALA at concentrations of 1000 µg/ml at 31.3 µg/ml in serial dilution with 4 h of incubation, were carried out using Biotable IrradLED 630 nm (10 J/cm², 40 mW/cm²) for irradiation, and for the PDZ, were used concentrations of 200 µg/ml at 6.3 µg/ml, serial dilution, with 1 h incubation, using Biotable IrradLED 660 nm (10 J/cm², 25 mW/cm²), for irradiation . A significant decrease ($p < 0.01$) was observed between the groups treated at the different concentrations and the control groups, with a 95.2% decrease in the concentration of 125 µg/ml ALA and 100% in all concentrations of PDZ, in the parameters used. ALA is widely used for the treatment of brain tumors, since it is a compound capable of passing the blood-brain barrier, however the PDZ is a good alternative, comparing the concentrations of 125 µg/ml and 100 µg/ml, respectively, of ALA and PDZ, the chlorine presented better results with shorter incubation time.

S3-53. Results of a Single visit PDT treatment for nodular basal cell carcinoma, superficial basal cell carcinoma and Bowen disease

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Photodynamic therapy (PDT) is one of the non-invasive treatments options for BCC, and Bowen disease (BD), aproved for two sessions one week apart. For the old people, coming back to hospital to perform the second part of the treatment immediately one week after may be very difficult because of their comorbidities and the distance from the hospital. This study evaluated the effectiveness of this PDT single visit protocol where the patient receive all the treatment in just one single visit; and evaluation of recurrence 6 months after. From January 2016 to September 2018, 227 nodular BCC less than 1 cm, 96 superficial BCC and 39 BD were treated. The lesions were debulked and taken for histological evaluation. Then, a 20% aminolevulinate(MAL) cream was applied and covered for 3 hours. After this, the lesion was illuminated for 20 minutes with a LED at 630 nm (delivered energy of 150J/cm2, 125 mW/cm2 irradiance). After the illumination the lesion received another amount of MAL cream and covered, but now for 1,5 hour. Then, it was illuminated with the same parameters. In the 30th day after treatment, a 2mm punch biopsy evaluated the response. The remained patients were evaluated after 6 months. Residual BCC was surgicaly removed. The 30th day biopsy showed 93% of complete response in 227 nodular BCC, 99% for superficial BCC and 85% of complete response for BD treated through this PDT single visit protocol. The analysis 6 months after PDT could be performed in 158 lesions of nodular BCC and showed only 6% of recurrence. For the superficial BCC the 6 months analysis could be performed in 69 lesions and a 4% of recurrence was observed. Considering the BD, the 6 months recurrence was observed in 16%, during the follow up of 19 lesions. This study demonstrated 93% of complete response for small nodular BCC, 99% for superficial BCC and 85% for BD.This new technique showed high rate of cure comparable to surgery, with advantages of aesthetics results and low costs.