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Contents

SPIE would like to express its deepest appreciation to the symposium chairs, conference chairs, programme committees, session chairs, and authors who have so generously given their time and advice to make this symposium possible.

The symposium, like our other conferences and activities, would not be possible without the dedicated contribution of our participants and members. This programme is based on commitments received up to the time of publication and is subject to change without notice.

Conference 9531: Biophotonics South America

Saturday - Monday 23–25 May 2015

Part of Proceedings of SPIE Vol. 9531 Biophotonics South America

9531-1, Session 1

Optical coherence tomography for tissue imaging *(Invited Paper)*

Arnaud Dubois, Lab. Charles Fabry (France)

No Abstract Available

9531-2, Session 1

Double Stokes Mueller polarimetry to reveal ordered molecular structures within biological tissue

Masood Samim, Serguei Krouglov, Virginijus Barzda, Univ. of Toronto (Canada)

Second order nonlinear optical signals are highly sensitive to the molecular symmetry and organization of molecules. The polarization dependent SHG microscopy investigations of biological structures provide critical information about the molecular organization in the tissue, which plays a major role in determining mechanical and functional properties of the biomaterials. For example, SHG polarization microscopy has revealed that collagen organization in lung tissue is affected by cancer. Similarly, hierarchical structures of collagen and myosin have been probed and characterized over a broad range of organizational structures, from molecular level to ultra-structures of fibers and tissue level. In an optical setup the polarization-dependent interaction of light with matter can be described using Stokes Mueller formalism. In the Stokes Mueller description, the light is represented by a four-element Stokes vector, and its interaction with matter is represented by a Mueller matrix. Stokes vector can describe interactions with partially polarized light and operates with intensities, which are real numbers and thus are observables in the experiment. The Jones formalism is frequently used to describe pure polarization states of laser radiation. However, the biological tissue is a highly heterogeneous scattering material; therefore, there is a benefit to employ Mueller Stokes formalism in the polarization analysis of the nonlinear optical responses. We first describe the theoretical background that underlies the Stokes Mueller polarization formalism for the two-photon processes. We will describe expressions for the light-matter interaction of second-harmonics generation using the double Stokes Mueller formalism that resembles the conventional linear form. We show a convenient expression for the double Mueller matrix components in terms of the nonlinear susceptibility tensor components, which are used to characterize nonlinear properties of the material. We will further discuss the properties of the nonlinear Mueller matrix and give the matrix expressions for cylindrically symmetric materials, which are frequently found in the biological structures. By performing nonlinear Stokes polarimetry measurements we show that it is possible to examine nonlinear properties of the collagen and myosin structures in the tissue and determine uniquely the double Mueller matrix components values. Finally, we will provide the expressions for double Stokes polarimetry using arbitrarily oriented linear polarization-in polarization-out (PIPO) measurement configuration. This information, for example, can be used in histopathology to characterize collagen structure in a cancerous tissue.

9531-3, Session 1

Time lapse microscopy of oxidative stress in lung endothelial cells

Mahsa Ranji, Zahra Ghanian, Univ. of Wisconsin-Milwaukee (United States); Ganesh G. Konduri, Medical College of Wisconsin (United States)

In this study, we have quantified the dynamics of oxidative stress in lung endothelial cells (from sheep model) using time-lapse microscopy. The intensity of the fluorescence images within the cells was measured over time (using our image cytometry tool) before and after cells treated with chemical perturbor containing potassium cyanide (KCN, inhibitor), or uncoupler such as pentachlorophenol (PCP). KCN and PCP both induced increased fluorescence intensity in the red channel with fluoresence tag of MitoSox, which is a measure of the mitochondrial oxidative stress. The results show that KCN and PCP significantly increased mitochondrial oxidative stress by (1.9812 ± 0.3556) and (18.1116 ± 1.9) times, respectively, when compared to cells not treated with mitochondrial perturbor. Our developed method provides a way to quantify the effect of the mitochondrial dysfunction in lung endothelial cells and the amount of injury sustained.

9531-4, Session 1

Delivery of ultrashort spatially focused pulses through a multimode fiber for two photon endoscopic imaging

Christophe Moser, Edgar E. Morales Delgado, Ioannis N. Papadopoulos, Salma Farahi, Demetri Psaltis, Ecole Polytechnique Fédérale de Lausanne (Switzerland)

Due to their high number of supported modes, multimode optical fibers carry large amount of spatio-temporal information. However, propagation of a light pulse through a multimode optical fiber suffers from spatial distortions due to superposition of the various exited modes and from time broadening due to modal dispersion. Here, we present a method based on digital phase conjugation to selectively excite specific optical fiber modes in a multimode fiber that follow similar optical paths as they travel through the fiber. In this way, they can be made to interfere constructively at the fiber output to generate an ultrashort spatially focused pulse. The excitation of a limited number of modes limits modal dispersion, allowing the transmission of an ultrashort pulse. We also show that the short spatially focused pulse can be scanned digitally without movable elements. We experimentally demonstrate that the pulse at the output of the multimode fiber generate a two-photon signal. We show delivery of a 1550 nm pulse with 720 fs duration, spatially focused to a spot size of 10 micrometers, through a 30 cm long, 200 micrometers core multimode fiber. We show how this technique is applied to endoscopic two-photon imaging.

9531-5, Session 1

Image correlation based method for the analysis of collagen fibers patterns

Ramon G. Rosa, Sebastião Pratavieira, Cristina Kurachi D.D.S., Univ. de São Paulo (Brazil)

The collagen fibers are one of the most important structural proteins in skin, being responsible for its strength and flexibility. It is known that several skin conditions modify collagen properties like fibers density, ordination and mean diameter. Therefore, a study of these characteristics can help on the diagnosis of such conditions. Usually, a microscopic study of these properties requires the use of selective dyes, what makes these methods unsuitable for in vivo imaging. However, the collagen structures lack of a center of symmetry. This gives rise to interesting nonlinear optical properties like a non-null second order susceptibility tensor, which allows phenomena like the Second Harmonic Generation (SHG). Non-centrosymmetric media are rare in biological tissues, thus the SHG microscopy is a powerful noninvasive tool for imaging collagen fibers. In addition, nonlinear microscopy techniques presents an intrinsic three dimensional resolution, causes less photodamage and achieves larger penetration depths when compared to standard laser scanning fluorescence microscopy. We analyzed the images through its two dimensional spatial Autocorrelation Function (ACF). The radial decay rate of the ACF was successfully related to the mean diameter of the fibers and the anisotropy of this decay allowed us to determine the anisotropy and the preferential orientation of the fibers in the image. By slicing the image in multiple parts, we could study the direction field of the fibers and analyze the evolution of the obtainable parameters over time. The analysis were done using the software MATLAB®.

9531-6, Session 1

Tissue characterization using polarization-sensitive second harmonic generation microscopy

Danielle Tokarz, Princess Margaret Cancer Ctr. (Canada); Richard Cisek, Ahmad Golaraei, Serguei Krouglov, Univ. of Toronto Mississauga (Canada); Roya Navab, Carolyn Niu, Princess Margaret Cancer Ctr. (Canada); Kazuhiro Yasufuku, Toronto General Hospital (Canada); Ming-Sound Tsao, Shingo Sakashita, Princess Margaret Cancer Ctr. (Canada); Sylvia Asa, Toronto General Hospital (Canada); Virginijus Barzda, Univ. of Toronto Mississauga (Canada); Brian C. Wilson, Princess Margaret Cancer Ctr. (Canada)

Changes in the ultrastructure of collagen in various tumor and non-tumor human tissues including lung, pancreas, thyroid and breast were investigated ex vivo by a polarization-sensitive second harmonic generation microscopy technique referred to as polarization-in, polarization-out (PIPO) SHG. This involves measuring the orientation of the linear polarization of outgoing SHG as a function of the linear polarization orientation of incident laser radiation. From the PIPO SHG data, the second-order nonlinear optical susceptibility ratio, ?(2)zzz/?(2)zxx, for each pixel of the SHG image was obtained and presented as color-coded maps. Further, the orientation of collagen fibers in the tissue was deduced. Since the ?(2)zzz/?(2)zxx values represent the organization of collagen in the tissue, theses maps revealed areas of altered collagen structure (not simply concentration) within tissue sections. Statistically-significant differences in ?(2)zzz/?(2)zxx were found between tumor and non-tumor tissues, which varied from organ to organ. Hence, PIPO SHG microscopy could potentially be used to aid pathologists in diagnosing cancer. Additionally, PIPO SHG microscopy could also aid in characterizing the structure of collagen in other collagen-related biological processes such as wound repair.

9531-7, Session Pln1

Light and life

Herch Moysés Nussenzveig, UFRJ (Brazil)

We revisit Niels Bohr's famous 1932 lecture with the same title, as well as his later pronouncements, confronting them with current knowledge. Topics Covered include: life origin and evolution, quantum mechanics and life, brain and mind, consciousness and free will, and light as a tool for biology, with special emphasis on optical tweezers and their contributions to nanobiotechnology.

9531-9, Session 2

Non-linear optical visualisation of DNA liquid crystal phases

Katarzyna Matczyszyn, Joanna Olesiak-Banska, Katarzyna Brach, Wroclaw Univ. of Technology (Poland); Marta Gordel, Wroclaw Univ. of Technology (Poland) and Ecole Normale Supérieure de Cachan (France); Jospeh Zyss, Ecole Normale Supérieure de Cachan (France); Marek Samoc, Wroclaw Univ. of Technology (Poland)

At sufficiently high concentration, DNA helices in aqueous environment have a tendency to self-organize into lyotropic liquid crystal (LC) phases [1-14]. Investigations of such structures are helpful for the understanding of biological processes such as in vivo packing of DNA in the cell nucleus or dynamic processes of DNA replication where the condensation of DNA is needed in order for the helices to be in close contact with each other. The condensation and re-condensation are actually processes of phase transitions in the LC phases of DNA. The aptitude to form LC phases even out of very short DNA helices (oligonucleotides) is nowadays seen as one of the features that facilitated the creation of life on earth [15]. On the other hand, the structuring of DNA into liquid crystal phases can be of interest for fabrication of meta-materials by incorporating nanoparticles, as was shown on the example of lyotropic lipid LCs containing gold nanorods [16]. One of the limitations for the use of DNA in fabrication of materials is solubility. Indeed, DNA is only soluble in water, whereas organic solvent compatible derivatives of DNA such as, for example, the DNA-CTMA complex [17], do not form LC phases.

Our technique of choice for imaging the organisation of DNA is polarisation sensitive two-photon excited fluorescence microscopy (ps-TPFM), originating from pioneering work of W. Webb [18] and implemented by us [19]

The only restriction of the resolution is the fluorescence of the dye staining DNA, whereas the advantages are numerous: limitation of photobleaching (as the dye molecules out of focus are not bleached), focused excitation "in point", the sample is excited with IR light, which causes less damage to the material and increases the penetration depth.

The imaging is performed via the observation of the fluorescence from the dye molecules as DNA itself fluoresces in the UV region, thus making direct observation inconvenient from the experimental point of view. Research on efficient fluorescent dyes with well-defined binding modes to DNA is very active [21-23], a major target being the stains excited in the "tissue window" from 800 to 1200 nm as those dyes can be applied to in vivo experiments where the light is not absorbed by tissues.

In our current studies we use also gold nanoparticles, mainly nanorods, to research both the possibility of exploiting nanostructures towards nonlinear imaging as well as their impact on DNA LC phases [20, 24, 25]. This lead us to investigate in depth the stability of LC phases and cholesteric and columnar phase transitions when doped with the gold nanoparticles.

We are working in the in vitro conditions but mimicking the real concentrations of DNA in cells, thus making our research of interest to biologists, while looking from a physic-chemical perspective at the interaction between dyes and nanoparticles with a DNA host. As gold nanoparticles are being used for cancer phototherapy, it is crucial to understand their behavior in such conditions.

9531-10, Session 2

EGFR-specific nanoprobe biodistribution in mouse models

Samia B. Fashir, Michael A. Hupman, Dalhousie Univ. (Canada); Maiara L. Castilho, Univ. do Vale do Paraíba (Brazil); Christopher L. D. Lee, Dalhousie Univ. (Canada); Leandro J. Raniero, Univ. do Vale do Paraíba (Brazil); Ian Alwayn, Kevin C. Hewitt, Dalhousie Univ. (Canada)

We created and studied a novel biocompatible nanoprobe for spectroscopic molecular imaging of the Epidermal Growth Factor (EGF) receptor, which is over-expressed on the cell surface of certain cancers. These nanoprobes exhibit SERS, when aggregated in solution, with the silver nanoprobes exhibiting the greatest enhancement. Spectroscopic detection at the picomolar level was demonstrated. They have the necessary properties – specificity, sensitivity and stability – to serve as molecular imaging agents. This contribution describes our effort to assess their biodistribution in animal models.

Mice share a high degree of homology with humans, being a close match in anatomy, physiology and genetics. Therefore studying the nanoparticle distribution in mice will provide insight into the nanoparticle distribution in humans.

Wild type C57BL6 mice were divided into control and experimental groups. Mice in the control group were injected with 100 ?L 0.9% saline solution. The experimental groups were injected with various treatments consisting of either gold or silver nanoparticles of similar sizes, treated with either alpha lipoic acid (non-specific) or MEGF + alpha lipoic acid (specific) as specified below. All injections are of equal volumes (100 ?L). Both gold and silver nanoparticles are used in this experiment to compare any difference in distribution.

Each animal was anesthetized by placing it in an induction box and delivering 2.5% Isoflurane with 0.8L/min medical air flow rate. Once anaesthetized the mouse was placed on it's side to allow access to the tail vein, preheated using a warming lamp for 3-5 minutes to further enhance visualization and vasodilation. The mouse was then injected with 100 ?L of solution as specified below (~10^10 NPs per injection). An hour after injection, a blood sample was collected from the mice via the saphenous vein. This was repeated at 4hrs, 12 hrs, 16 hrs, 20hrs, and 24 hrs post injection. Blood was collected by first heating the mouse and shaving the back of the hind leg with electric clippers so the saphenous vein is visible. The mouse was then placed in a centrifuge tube to restrain the body while the hind legs are immobilized in the extended position by applying slight pressure above the knee joint. Using a 23 gauge needle the tail vein was punctured to collect blood directly into collection tubes.

To collect the additional samples, the blood clot formed at the puncture site is removed. In the case of insufficient volumes of blood collected from saphenous veins, additional samples were collected from the tail vein.

Urine samples were collected by removing the mice from their cage and placing them in a large cardboard box covered with layers of bench coat, directly on top a layer of glad cling wrap. The structure will be placed on top of the cling wrap and and animal is placed in each of the compartments. The animals are allowed to urinate freely and the urine collected using a pipette. Feces samples were collected from the cage when feasible. After the 24 hr mark the mice are euthanized via CO2 overdose while under general anesthesia (inhaled isofluorane). The liver, spleen, kidney, heart, pancreas, brain, stomach, bowels, skin, and lungs were harvested. The organs were then rinsed with PBS buffer (physiological saline) and stored in pre-labeled tubes, weighed, homogenized and stored in the freezer (-80 degree C) and sent for inductively coupled plasma mass spectrometry (ICP-MS).

The liver, spleen and bowels show the greatest uptake, at levels (per mass of tissue) of 4x10^-7 for AgNPs coated with alpha lipoic acid, 1.5 x 10-7 for AgNPs coated with MEGF, and at 2X10^-8 for AuNPs coated with MEGF. Comparing gold and silver nanoparticles coated with MEGF, an order of magnitude reduction in uptake, with the latter being near background levels. The Au-MEGF nanoprobes appear to avoid an immune response. Results from the blood, urine and feces analysis will be presented at the conference to confirm these very important findings.

9531-11, Session 2

Beyond the diffraction limit: subwavelength biophotonics and nanofluidics

Ahmet A. Yanik, Univ. of California, Santa Cruz (United States)

In this talk, I will introduce a number of new technologies based on nanoscale control of light and fluidics on a chip. I will show how to overcome some of the fundamental limitations of the state of art techniques used in vitro diagnostics, which makes up a $10B global market.

An ultrasensitive detection technology with detection limits surpassing the gold standard surface plasmon resonance (SPR) sensors will be introduced. Practical applications of this technology allowing million fold enhanced multiplexing capabilities with minute sample volumes will be shown. Using a bottom-up approach, I will merge nanofluidic and nanophotonic technologies at sub-wavelength dimensions to break the diffusion barrier. For point-of-care applications, a novel sensing scheme enabling label-free detection of biomarker proteins “with the naked eye” will be introduced. Real world applications of these optofluidic-plasmonic sensors for rapid and reliable detection of whole viruses (such as Ebola) from biological media will be demonstrated. I will extend this nanofluidic approach to a micro-nano cross flow scheme for isolation of rare circulating tumor cells (CTCs) for cancer diagnostics and prognosis. Potential implications of this technique for the analysis of single tumor cells and their metastatic potential will be discussed.

9531-12, Session 2

Nanoparticle-based contrast agents for dental optical coherence tomography

Ana K. S. Braz, Renato E. de Araujo, Univ. Federal de Pernambuco (Brazil); Guanying Chen, Tymish Y. Ohulchanskyy, Earl J. Bergey, Shobha Shukla, Univ. at Buffalo (United States); Anderson S. L. Gomes, Univ. at Buffalo (United States) and Univ. Federal de Pernambuco (Brazil); Paras N. Prasad, Univ. at Buffalo (United States)

Optical coherence tomography (OCT) is a well-established, low-coherence interferometric technique that performs high-resolution, noninvasive, and cross-sectional tomographic imaging of tissue microstructures. Moreover, the use of contrast agents could lead to the enhancement of OCT image quality to a degree comparable to biopsy, reducing the need to remove, section, and stain tissues. Here, we demonstrate the potential use of spherical and core-shell nanoparticles as contrast agents for OCT in dentistry. On the formation of spherical nanoparticles, gold precursor was introduced in commercial dental bonding adhesives and by photothermal reduction gold nanoparticles were generated in situ. The presence of gold nanoparticles inside the hybrid layer and dentinal tubules was identified on OCT images. The diameter of the gold nanoparticles was determined to be in the range of 40 to 120 nm. Moreover, 150 nm diameter TiO2 coated fluoride core-shell nanoparticles was also introduced in the tooth, by the use of a commercially available dental bonding agent. The core-shell nanostructures were explored on the evaluation of the dental bonding structure. By using OCT we analyzed the presence of nanoparticles at depth and in a non-destructive way. A commercial optical coherence tomography system was used to produce cross-sectional images. This system can generate 29 frames per second with 512 lines per frame and an axial resolution of 5 ?m. We showed that nanoparticle-based contrast agents for OCT allow better image quality, improving the identification of tooth structures, and therefore extending the application of this technique to many diagnostic procedures.

9531-13, Session 2

Optoacoustic spectroscopy measurements of water-soluble iron oxide nanoparticles

Herve K. Nguendon Kenhagho, Daniel C. Gallego, Univ. Carlos III de Madrid (Spain); Leisha M. Armijo, Yekaterina I. Brandt, Marek Osi?ski, The Univ. of New Mexico (United States); Horacio R. Lamela Rivera, Univ. Carlos III de Madrid (Spain)

Different sizes of Fe3O4 nanoparticles used as contrast or drug delivery agents gained substantial interest due to their intrinsic superparamagnetic properties and low cytotoxicity. The Fe3O4 nanoparticles have strong light absorption in the visible and near infrared range, overlapping the biological transparent window of 650 - 950 nm. The contrast between various tissues can be extracted based on the diffuse optical spectroscopy technique and used for example in breast cancer diagnostics, although the optical system needs highly sensitive optical sensors. One optoacoustic (OA) spectroscopy method relies on the amplitude of the generated acoustic transients, which is directly proportional to the optical absorption at each wavelength (Lamela et al. 2008, Proc. SPIE 6856).

Our previous studies have been in optical and OA measurements of the absorption properties of gold nanoparticles within a highly scattering medium. The studies were performed by using a laser OA scheme with a Nd:YAG laser pumping an OPO system with a pulse energy of millijoules, a pulsewidth of 5 ns, a tunability range between 410-2400 nm, and a repetition rate of 10 Hz.

In this work, we will present new experimental results on intrinsic optical properties (absorption and scattering coefficients) of Fe3O4 nanoparticles obtained using a high energy short-pulse multispectral laser source, and will compare these results with our earlier studies on gold nanoparticles. The spectral range covered by the system is from 410 nm to 1700 nm, which represents an extension further into near infrared with higher sensitivity than our previous experimental setup. The wavelength extension allows us to investigate the nanoparticle properties further towards infrared, which is of high interest for diagnostic and therapeutic applications.

9531-14, Session 2

In vivo luminescence imaging and tomography using upconverting nanoparticles as contrast agents

Stefan Andersson-Engels, Monirehalsadat Mousavi, Hugo Söderlund, Lund Univ. (Sweden); Haichun Liu, National Univ. of Singapore (Singapore)

Imaging is becoming increasingly important for tissue biology, as it provides possibilities for minimally invasive monitoring of molecules and molecular interactions in vivo. It enables visualization of basic patho-physiology reactions and to follow them over time. Imaging is also frequently used for drug discovery and evaluation, by providing possibilities for in vivo pro-drug screening in longitudinal studies. Optical imaging is the simplest and most cost effective of the molecular imaging modalities and thus a very valuable tool. Luminescence, the most sensitive of the optical imaging techniques, is by far the most frequently used. It has though some limitations for imaging deep structures due to high light scattering and attenuation, and is thus best suited for small animal imaging.

Upconverting nanoparticles (UCNPs) have been suggested as an improved contrast agent for luminescence small animal molecular imaging, as it provides a tissue autofluorescence-free signal, improved spatial resolution and depth sensitivity. Normally NaYF4:Yb3+,Tm3+@ NaYF4 UCNPs have been employed in such studies. We now quantitatively explore suggested improvements in UCNPs imaging to further improve the potential for sensitive imaging deeper into tissue. The first improvement is to employ Nd3+-codoped UCNPs to enable excitation at a wavelength that is less attenuated by water molecules in tissue. This provides a ten-fold signal increase for deep tissue structures. The other is to modify the optical arrangement as typically employed to fully explore the optical filter performance, again improving the signal-to-background by up to ten-fold for signals deep into tissue.

9531-15, Session 2

Plasmonic cell transfection using micropyramid arrays

Nabiha Saklayen, Marinus Huber, Daryl I. Vulis, Harvard Univ. (United States); Marinna Madrid, Harvard School of Engineering and Applied Sciences (United States); Valeria Nuzzo, ECE Paris (France); Eric Mazur, Harvard School of Engineering and Applied Sciences (United States)

We present a new cell transfection method that uses femtosecond laser-excited localized surface plasmons (LSPs) on a nanostructured micropyramid array. Our gold-layered micropyramids have nano-apertures at the apex to form high local electric field enhancements, or “hot spots.” These hot spots form microbubbles that temporarily perforate mammalian cell (HeLa S3) membranes and allow dye molecules and plasmid vectors to diffuse through the membrane openings. We introduce an emerald green fluorescent protein (EmGFP) reporter plasmid into the cells to determine the LSP-mediated transfection efficiency of the substrate. We fabricate large-scale pyramid arrays using photolithograpy and optimize our laser parameters for successful transfection and high cell viability. Our nontoxic, efficient, and scalable technique offers an innovative approach to the advancement of regenerative medicine and the study of LSP-cell interaction.

9531-63, Session PSat

Comparative clínical study using laser and led-therapy for orofacial pain relief: dental hipersensitivity and cervicogenic headache

Rosane F. Z. Lizarelli, NILO - Nucleo Integrado de Laser em Odontologia (Brazil); Renata C. A. Pizzo, Jose Geraldo Speciali, Vanderlei S. Bagnato, Univ. de São Paulo (Brazil)

The low intensity laser therapy has been widely applied in pain relief or analgesia mechanism considering several clinical situations. With the advent of new LED-based (light emitting diode) light sources, the need of further clinical experiments aiming to compare the effectiveness among them is paramount. The LED system therapeutic use can be denominated as LEDT – Light Emitting Diode Therapy. This study proposes two clínical evaluations of antialgic effect: to dentin hypersensitivity and to cervicogenic headache using different sources of lasers (low and high intensity) of light emitting diodes (LEDs), one emitting at the spectral band of red (630 +/- 5 nm) and the other one at infrared band (880 +/- 5 nm). Considering dentin hypersensitivity, red and infrared led were so effective than the control group (high intensity laser system); for the other side, consideting cervicogenic headache, control group (infrared laser) was the best result in comparison to red and infrared led system.

9531-64, Session PSat

Studying of light scattering in aqueous samples should collagen in curcuma pigments presence and nanoparticles

Francisleia Maria L. Silva, Cláudia Adriana Sousa Melo, Cleânio da Luz Lima, Univ. Federal do Piauí (Brazil)

Today the nanotecnologia is a much used technology for various purposes. The industry cosmetic have invested much in nanotechnology because the nanoparticles penetrate layers tissue deeper than traditional products therefore products having nanoparticles are most products efficient. The purpose of this work is to study as the light scattering in the biological tissue if alter in the presence of nanoparticles and pigments. This work seeks to accomplish a comparative study the light scattering in the presence or no of nanoparticles and pigments. For accomplish this study we seek first simulate a tissue biological using a collagen aqueous then we adicionamos nanoparticles and pigments in these samples. We mount a experiment for analysis of the samples of form that obtain the profile of light scattering in the samples. for mount of the experiment was used one laser incident perpendicular to the sample, one camera CCD was positioned to capture the light scattering picture in the sample. The pictures obtained was analyzed utilizing the software ImageJ the gray levels in each pixel of the picture. The obtained result give the decay of the light intensity in the samples different. With the obtained result we can behavior compare the samples differents analysed.

9531-65, Session PSat

Methylene blue photodynamic therapy in rats wound healing : 21 days follow-up

Vanda S. Carneiro, Marleny E. M. Gerbi, Univ. Federal de Pernambuco (Brazil); Maria Helena C. V. Catão, Univ. Estadual da Paraíba (Brazil); Natália C. Araújo, Rebeca F. Menezes, Univ. Federal de Pernambuco (Brazil)

The experimental evaluated the photodynamic therapy (PDT) in wound healing. It used 48 male rats, making two circular wounds at each animal. They were treated at 48hs intervals, with methylene blue (MB), low level laser treatment (LLLT) or both, thus resulting in PDT. The wounds were observed 01, 03, 07, 14 and 21 days after and then processed and subjected to HE staining to analyze granulation tissue, necrosis, epithelialization and collagen. After day 1, wounds treated with MB showed necrosis less intense than other groups, and the PDT group showed more intense granulation tissue. At day 3, reepithelialization was absent for half of injuries in the PDT group, and this group was also with lower collagen. However, at day 7, this same group presented reepithelialization more advanced than control group, which did not happen with those treated with MB or LLLT ($p = 0.015$). The results allow us to conclude that PDT difficulted reepithelization at 7th day and interfered in standard healing. However, when used separately, MB and LLLT interfered significantly compared to the control group, which did not happened to the PDT group. There was no significant difference between the treatment groups in other analysed times.

9531-66, Session PSat

Clinical study on orofacial photonic hydration using phototherapy and biomaterials

Rosane F. Z. Lizarelli, Natalia DelPadre Grandi, NILO - Nucleo Integrado de Laser em Odontologia (Brazil); Clovis Grecco, Univ. de São Paulo (Brazil); Luciana Almeida-Lopes, NUPEN - Nucleo de Pesquisa e Ensino de Fototerapia nas Ciencias da Saude (Brazil)

Skin hydration is important to prevent aging and dysfunction of orofacial system. Nowadays, it is known that tegumentar system is linked to muscle system, then every dentist need to treat healty facial skin, as lips, keeping orofacial functions healthy. Thirty-two patients were treated using laser and ledtherapy single or associated to biomaterials (dermocosmetics) searching for the best protocol to promote skin hydration. Using a peace of equipment to measure eletric impedance, percentage of water and oil from skin, before and after different treatments were analized. Statistic tests using 5% and 0.1% of significance were applied and results showed that light can improve hydration of epiderm layer of facial skin. Considering just light effect, using infrared laser followed by blue led system is more effective to hydration than just blue led system application. Considering dermocosmetic and light, the association between both presented the best result.

9531-67, Session PSat

Study of near-infrared spectroscopy in porcine mandible focusing to characterize porosity inside bone

Monique A. Nascimento, Aya Sugiura, Patricia A. Ana, Emery C. Lins, Univ. Federal do ABC (Brazil)

Near-Infrared Radiation Spectroscopy (NIRS) is a technique that detects infrared radiation backscattered out from tissue due to its own optical properties and anatomy, revealing characteristics of tissue deeply. This article reports initial results of NIRS applied on porcine jaws aiming to characterize porosity inside bone. The specimens were three cleaned mandibles of pig, free from skin and oral mucosa. A 3W lamp-based (350-2200nm) light source with a fiber optic illuminated the samples, while a spectrometer with an InGaAs sensor (900-1700nm) captured NIR spectra. The method consist of measure ten spectra of the backscattered radiation at each point of investigation and average them to one spectrum. We selected points of investigation displaced 2,0mm, 5,0mm and 10mm far from illumination. Initial result from one specimen reveals radiation intensity at 1300nm in a massive site is 40% lower than intensity in a porous site. Additional results and analysis will be presented soon.

9531-68, Session PSat

Optical proprieties of human radicular dentin: ATR-FTIR characterization

Jose Quinto, Instituto de Pesquisas Energéticas e Nucleares (Brazil) and FMU Laureate (Brazil); Claudia B. Zamataro, Derly A. Dias, Carolina Benetti, Denise M. Zezell, Instituto de Pesquisas Energéticas e Nucleares (Brazil)

The knowledge of dental structures in details is helpful to understand and have better the interaction with dental materials. For example the adhesion plays a very important role for dentistry . The dentinal tubules are the basic unit for human dentin. The density range from 4.900 to 90.000 tubules per mm2, and diameter from 1 to 3 μmi. The light propagation inside the tubules is associated due to tubules orientation were can be found light amplification. The use of laser in dentistry is a routine and it may be affected trough this effect, but as far as it is known, there is no publication yet demonstrating the physical-chemical

alterations in the dentin. The Er,Cr:YSGG (?= 2,79 ?m) laser used in this study (energy density 9,46 J/cm2) was above the ablation rate.

The ATR-FTIR (2000 to 700 cm-1) was used to evaluate the influence of dentinal tubules orientation due to laser bean and correlate the effect when the tubules are in same orientation or perpendicular. At this region there are the range of interest in dentinal tissue. It was one target also analyze the changes that occurs in different areas of the root canal, as long as there are differences in density of tubules among each root third location and optical density variations in the intertubules region (n=1,45), peritubules (n=1,65) and intertube solution (n=1,33) . It was observed differences among region and tubules orientation due to irradiation beam direction.

9531-69, Session PSat

Blue led irradiation to hydration of skin

Priscila C. Menezes, Rosane de Fátima Zanirato Lizarelli, Michelle B. Requena, Vanderlei S. Bagnato, Univ. de São Paulo (Brazil)

Blue led system irradiation has some important properties to skin: contamination reduction, bleaching and biostimulation. However, clinically, it is easy to realize that blue led light can promote skin hydration. As there is not some paper about that, it is mandatory study and confirm this clinical indication. Ten patients were selected to this study with age between 25-35 years old, presented phototype I, II and III. A defined area from forearm was pre-determined (A = 4.0 cm2) and we have used a peace of equipment with blue LED under power of 5.3mW and irradiance of 10.8mW/cm?. We have choosen two different doses: 3 J/cm2 and 6 J/cm2. Under corneometric measures, patients were analized on 7, 14, 21 and 30 concerning about porcentage of water into skin. Statistical test of ANOVA, Tukey and T-Student were applied considering 5% of significance. In conclusion, both doses were able to hydrate skin, however, 6J/cm2 has kept this hydration for 30 days.

9531-70, Session PSat

Excited stated dynamics in self-assembled photosensitizer films

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Porphyrins, chlorins and their derivatives have been extensively studied since the discovery of their potential application as photosensitizers (PSs) in photodynamic therapy (PDT). In the present work, self-assembled chlorin films are used as a model system for studying how chlorin surface density and arrangement affect processes associated to excited state migration via energy transfer between chlorin molecules as well as to the rate of triplet oxygen formation. Layer-by-Layer (LbL) methodology combined Spin-coating (SC) technique was used to self-assembling bilayers containing chlorin derivatives as anionic material and cationic polyelectrolytes, both sequentially deposited on inert quartz substrates. Absorption spectroscopy and Confocal Laser Scanning Microscopy (LSCM) imaging were used in this investigation in order to quantify both molecular adsorption and film homogeneity. Both methodologies confirmed that chlorin bilayers are homogeneous and transferred consistently to the quartz surface. Moreover, the chlorin packing process produces a blue-shift in the fluorescence when compared with chlorin solution. Fluorescence decay time decrease substantially due to the special molecular packing assumed by chlorin molecules in the LbL films and fluorescence lifetime imaging (FLIM) confirmed the homogeneity of this molecular arrangement along the substrate surface. Photophysical studies in order to determine the singlet oxygen quantum yield of LbL chlorin films in water solution and how they interact with other molecular systems will be presented.

9531-71, Session PSat

Efficacy of the photodynamic inactivation of oral microorganisms with the use of curcumin associated to blue LED (λ450nm±5nm): in vitro study

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It has been suggested that curcumin has antimicrobial effects which can be exacerbated with light at a wavelength appropriate. The aim of this study was to evaluate the effect of lethal photosensitization (LP) mediated by blue LED associated to Curcumin with the parameters: λ450nm ± 5nm, 220mW and spot of 0.785 cm^2. Microorganisms from the oral mucosa and the posterior region of the tongue were collected from three individuals and inoculated into test tubes containing 8 mL of culture medium followed by TSB. Three culture dishes of 24 wells were used. To each well was added 400μL of the suspension containing the microorganisms. Suspensions without curcumin were placed in eight wells. Elsewhere, curcumin was applied varying concentrations of 75, 37.5, 18.75, 9.37 and 4.68 mg / mL and a pre-irradiation time of 5 min was awaited. Irradiated wells, the LED was applied on the samples by 3'37". After stirring, 200μL were removed from each well was made and immediately read by the ELISA method, for turbidity and/or by colorimetry. After 1 hour incubation in an oven bacteriological other 200μL were removed from the remaining wells to a second reading. It was observed that the concentration of 75 mg / ml showed the best result, and therefore the tests were repeated and the final results indicated an immediate result for the favorable concentration of 75 mg / mL (81%). Thus, this study suggested that FL with blue LED associated to Curcumin could be a potential mechanism for controlling the infection of the oral microorganism.

9531-72, Session PSat

In vitro evaluation of the efficacy of lethal photosensibilization of oral microorganisms with Photogen associated to red LED (λ640nm±5nm)

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The aim of this study is to evaluate the lethal photosensibilization of microorganisms mediated by red LED (MMOptics, São Carlos, SP, λ640nm±5nm, output of 120mW, spot of 0,785cm2) associated with Photogen. Microorganisms of the posterior dorsal region of the tongue and oral mucosa were collected and inoculated in 8mL of TSB medium overnight followed by inverse homogenization. Culture plates with 24 wells were used for the irradiated and non-irradiated species. Each well received 400μL of the suspension containing the microorganisms. In eight wells no Photogen was used and they were the irradiated and non-irradiated control. The remain of the wells had Photogen applied with a pre-irradiation time of 5 min in concentrations of 40, 20, 10, 5 e 2.5μg/mL. LED was applied for 5 min and 45 sec. After agitation, 200μL were withdrawn from each well and ELISA colorimetric and/or turbidity measurements were immediately taken. Other 200μL were withdrawn from the wells after 1 hour in bacteriological incubator for a second ELISA measurement. The best results found were for 40μg/mL Photogen associated with LED irradiation and immediate ELISA measurement (36.7%) and for 5μg/mL with LED irradiation with measurements one hour after incubation (42.8%). This study suggests that lethal

photosensibilization with Photogen associated to red LED can be a potential mechanism of control of oral microorganisms.

9531-73, Session PSat

Evaluation of the effectiveness of photodynamic therapy in the treatment of endodontic deciduous teeth: controlled clinical trial

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The elimination of pathogenic microorganisms in the root canal system is one of the main points in order to have success in endodontic treatment. The objective of this study is to conduct a randomized controlled clinical trial to evaluate the effectiveness of photodynamic therapy in endodontic treatment of deciduous teeth radiographical, clinical and microbiological. Thirty primary teeth will be selected of children aged between 3 and 6 years. The teeth will be randomly divided into two groups, Group I, control, conventional endodontic treatment and group II the endodontic treatment with application of photodynamic therapy will be performed. In both groups will be conducted microbiological analyzes that will be done before and after endodontic treatment and clinical and radiographic evaluations will be heldon 1 month, 3 months, and 6 months after treatment, and the comparison of the treatment in both groups will be held.

9531-74, Session PSat

Influence of collection geometry in the fluorescence collected by optical fibers in turbid media

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Scattering is one of the main optical properties interfering with light propagation in turbid media, this study aims to verify if different optical fiber tips interfere with the information collected by them when light propagates in a turbid medium. Two different optical fibers were compared: a flat-tipped optical fiber and a isotropic, spherically shaped tip optical fiber; the very different shapes of the tips allow for comparison regarding the collection geometry. A laser light source was delivered to a fluorescent center (a polymeric 0.3 mm diameter sphere), and the fluorescence was collected using both optical fibers for air and for a simple liquid phantom composed by powdered skinny milk. The fluorescence of the center was obtained for different positions by scanning the phantom volume, and the decay curves were plotted and compared to verify how close is the recovery of the attenuation coefficient of the same phantom composition for both collection geometries. The results shows that there is a great interference in light collection for the different optical fibers under the exact same conditions, and the attenuation coefficients obtained by both are quite different (e.g., 0.78 cm-1 for the isotropic optical fiber versus 1.80 cm-1 for flat-tipped optical fiber). These results are evidence of the importance in considering the effect of the collection geometry for optical properties assessment in biological tissues, and the consequences of diagnostic and therapeutic processes which applications are based on those parameters.

9531-75, Session PSat

Widefield fluorescence imaging and photodynamic therapy in a single LED-based device

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Photodynamic therapy (PDT) is a treatment modality that can be indicated for several cancer types and pre-cancer lesions. One of the main applications of PDT is the treatment of superficial skin lesions such as basal cell carcinoma, Bowen's disease and actinic keratosis. Three elements are necessary in PDT, a photosensitizer (PS); light at a specific wavelength to be absorbed by the PS; and molecular oxygen. A typical PS used in skin lesion is protoporphyrin IX (PpIX), which is an intrinsic PS and its production is stimulated by a pro-drug, such as Methyl-aminolevulinate (MAL) or 5-aminolevulinic acid (ALA). Before starting a treatment, it is very important to follow up the PpIX production (to ensure enough PS for PDT) and, during a PDT session, to monitor its photodegradation (as it is evidence of the photodynamic effect taking place). The aim of this paper is to present a unique device, LINCE (MMOptics - São Carlos, Brazil), that brings together two probes that can, respectively, perform fluorescence images and work as a light source for PDT treatment. The fluorescence imaging part of the system is optically based on 405 nm LED (light emitting diodes) arrays that allows observing the fluorescence emission over 450 nm. The PDT illumination probe is constituted of 630 nm LED arrays. Joining both functions at the same device makes PDT treatment simpler, properly monitorable and, hence, more clinically feasible. LINCE has been used in more than 1000 PDT treatments of superficial skin lesions in Brazil with excellent results.

9531-76, Session PSat

Asymmetry and irregularity border as discrimination factor between melanocytic lesions

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Image processing tools have been widely used in systems supporting medical diagnosis. The use of mobile devices for the diagnosis of melanoma can assist doctors and improve their judgment of a melanocytic lesion. This work proposes a method of image analysis for melanoma discrimination from other types of melanocytic lesions, such as regular and atypical nevi. The process is based on extracting features related with asymmetry and border irregularity. It were collected 104 images — from medical data — from two different databases during two years. The images were obtained from standard digital cameras without lighting and scale control. Metrics relating to the characteristics of shape, asymmetry and curvature of the contour were extracted from segmented images. Linear Discriminant Analysis was performed for dimensionality reduction and data visualization. Segmentation results showed good efficiency in the process, with approximately 88:5% accuracy. Validation results showed accuracy of approximately 70% for melanoma detection.

9531-77, Session PSat

Low intensity laser can decrease the mitochondrial activity of tumor cells

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Patients who have undergone oncological treatment with radiotherapy and chemotherapy for head and neck cancer show frequently mucositis, which is an inflammation of the mucous membranes lining the digestive tract, causing pain and compromising the cancer treatment. The low level laser therapy (LLLT) has been used successfully to reduce the clinical manifestations in the oral mucosa, however there is still some controversy regarding the effects of this irradiation on tumor cells that may remain after cancer treatment. The aim of this study was to analyze the effect of LLLT in recommended parameters for mucositis treatment regarding the mitochondrial activity of epidermoid carcinoma cells (strain SCC9). The cells were cultured in DMEM/F 12 supplemented with fetal bovine serum and hydrocortisone. The cultures were irradiated with 11 dosimetric parameters (wavelength 780nm and 660nm and power density between 118 to 283mW/cm?). Non-irradiated cultures served as control. Three independent experiments were performed in triplicate. After 1, 3 and 7 days of incubation, the cells (1x104) were submitted to the MTT assay. The mitochondrial activity of the irradiated cells were lower than the cells from the control group in all parameters tested during the experimental trial, except for cells treated with the wavelength of 660nm with an effective power density of 157mW/cm? that on day 7th showed the same behavior of the cells in the control group. The LLLT tested in 11 parameters were able to inhibit mitochondrial activity from tumor cells, which would demonstrate its safety regarding the mucositis treatment.

9531-78, Session PSat

Puchellin intracellular trafficking process studied by fluorescence correlation spectroscopy

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Fluorescence correlation spectroscopy (FCS) is one of the many different modes of high-resolution analysis of extremely low concentrated biomolecules. It has become a powerful and sensitive tool in fields like biochemistry, biophysics and biophotonics. As a well established technique, it is used to measure local concentrations of fluorescent labeled biomolecules, diffusion coefficients and kinetic constants. Pulchellin proteins (Abrus Pulchellus) are known by its high toxicity due to its ribosome-inactivating properties. They consist of a toxic A-chain linked to a sugar binding B-chain (type 2). Studies with mice and HeLa cells demonstrate that PII isoform is more toxic than PIV isoform. In our study we use commercial dyes (Alexa Fluor Dyes) linked to PII and PIV isoforms of pulchellin proteins. These labeled proteins were introduced in HeLa cells immersed in PBS medium. Through the FCS technique, available in the setup of the confocal microscope, it was possible to measure diffusion rates and then to monitor the intracellular uptake and traffic of PII and PIV pulchellin proteins. With the confocal facilities, we can choose a specific point inside the cell and monitor the change of protein concentration and the different diffusion rates during experiment. Our results demonstrate that PIV has faster cellular uptake compared to PII. We can also estimate the protein local concentration, in nanomolar range, due to the single molecule characteristics inherent to FCS.

9531-79, Session PSat

Raman spectroscopy analysis of highwavenumber region of oral squamous cell carcinoma and oral dysplasia

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Raman spectroscopy can provide a molecular-level of the biochemical composition and structure of cells with excellent spatial resolution and could be useful to monitor changes in composition for early stage and non-invasive cancer diagnosis, both ex-vivo and in vivo. In particular, the fingerprint spectral region (800–1,800 cm-1) has been shown to be very promising for optical biopsy purposes. However, fluorescence is often present and appears to affect the mid- IR/Raman region more than the high-wavenumber region (2,800–3,600 cm-1) Also limitations for discriminate dysplastic, and inflammatory process still persists in the fingerprint region. In addition, the Raman spectral signal of dysplastic cells is one important source of misdiagnosis of normal versus pathological tissues. In this way, we can use the high wavenumber spectral region in order to identify the subtle changes which could be important for discriminate the samples. In this study, we demonstrate the potential of high-wavenumber spectral region by collecting Raman spectra of nucleoli, nucleus and cytoplasm from oral epithelial cancer (SCC-4) and dysplastic (DOK) cell lines and from normal oral epithelial primary cells, in vitro, which were then analyzed by area under the curve as a statistical method to discriminate the spectra. In this region we will show the discrimination comparing the vibrational modes of CH3/ lipids and OH-confined water bands. This technique may provide a rapid diagnostic method and have potential use in early and non-invasive diagnosis of oral cancer.

9531-80, Session PSat

Optical coherence tomography analysis of prevention of dental erosion in primary teeth

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The aim of this study was to evaluate the efficacy of toothpastes containing sodium fluoride (NaF) and casein phosphopeptides (CPP)-amorphous calcium phosphate (ACP) regarding their potential to inhibit enamel erosion on primary teeth. Sixty 4?4 mm enamel specimens were randomly allocated into 4 groups (n=10): Negative control; Pronamelfor children (NaF); MI Paste Plus (CPP-ACP/NaF); Tooth Mousse (CPP-ACP). Erosive cycles with 0.5% citric acid, 5x, 3 min/day for 7 days were performed. After the first and last cycle of each day, toothpaste slurries was applied for 2 min. The quantitative analysis was accomplished using Optical Coherence Tomography (OCT).The use of CPP-ACP/NaF toothpaste was the most effective method for reducing mineral loss (77,5 %), followedbytheNaFtoothpaste (72,5%) and CPP-ACP toothpaste (55%).There was only significant differences between CPP-ACP/NaF and CPP-ACP toothpastes (p=0,044).Using the noninvasive OCT imaging technique, we quantitatively measured mineral loss, and identified the most efficient toothpaste among three employed that was effective in

erosion reduction. This exemplifies yet another application of OCT in a clinical environment.

9531-81, Session PSat

Biostimulation of the membrane protein Na, K-ATPase by low intensity laser

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The Na,K-ATPase is an active cation transporter protein that is found in the plasma membrane of virtually all animals cells. The electrochemical gradient generated by the protein allows the intracellular exchange of metabolites, nutrients, ions and regulate the cell volume. Therefore, the unregulated functioning of Na,K-ATPase is related to several diseases as cardiac arrhythmia, hypertension, renal and lung diseases, diabetes, and Alzheimer's diseases. Membrane bound Na,K-ATPase was obtained from the dark red outer medulla of the kidney of adult New Zeland white rabbits (Oryctolagus cuniculus) and protein sample was prepared in two different microenvironment: membrane fraction and reconstituted in DPPC:DPPE liposome. We irradiated the Na,K-ATPase samples by using three low-intensity lasers with wavelengths within the "therapeutic window" 532 nm, 650 nm and 780 nm.

The Na, K-ATPase enzymatic activity present in the membrane-bound fraction and reconstituted in liposome under irradiation had similar results, presenting an activity stimulation up to 15%, the variation of the enzymatic activity depends upon the amount of energy dose deposited showing stimulation and inhibition regions and the effect was independent (or subtle dependence) of the wavelength in the studied range. Moreover, we investigated the stability of the biostimulation and the results showed that the enzymatic activity returns to the basal level after 6 hours suggesting that the stimulation is not an oxidative process and it is reversible. These data and analysis may be useful to understanding the clinical effects of the low laser intensity therapy (LLIT)

9531-82, Session PSat

Detection and analysis of tooth wear using laser speckle images

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The analysis of laser speckle images in the spatial domain is a powerful tool capable of quantify micro-shifts at the ultrastructural level on tooth enamel. Speckle imaging is the underlabeling of periodic structure of the tooth that instead of appearing as a continuous and uniform structure, it appears as a discrete set of speckles due to the distribution of the teeth components. We observed that with the increasing of the acid exposure, the enamel hardness decreases and the relative ratio of the laser speckle contrast map proportionally increases. For the artificial wear, the samples were immersed in 30 ml of a cola-based beverage (pH 2.5) at room temperature. Immersion was performed twice a day over seven consecutive days during 10 min, 20 min, 30 min and 40 min. These experimental groups presented a shift in the LASCA maps of 18%, 23%, 39% and 44 %, respectively, demonstrating that these patterns are strongly correlated to the progressive tooth wear due to chemical processes.

9531-83, Session PSat

Attenuation coefficient of light in skin of BALB/c and C57BL/6 mice

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Optical properties of the biological tissueplay an important role to a correct use of optical techniques for therapy and diagnosis. The mice skin presents morphological differences due to characteristics such as gender, melanization, body mass and age. Murine models are frequently used in pre-clinical trials in optical therapy and diagnosis. Therefore, the assessment of skin tissue in animal models is needed for a proper understanding of how light interacts with biotissue. Noninvasive techniques such as optical coherence tomography (OCT), have been used to obtain optical information of the tissue, as the attenuation coefficient, with the advantage of obtaining sectional images in real time. In this work,eight female BALB/c albinomice (twenty-four weeksold)and eight male C57BL/6black mice (eight weeks old)wereused to measure theattenuation coefficient of light in the skin, utilizing the OCT technique, aiming to check for influence of body mass and age. Two moments were assessed, twenty-two weeks apart from each other. Ourdatasuggest that skin of albino mice possess attenuation coefficient higher than black mice, however, 22 weeks after, no statistical significant differences were observedintragroup.We conclude that light attenuation coefficient of mice skin may be influenced by factors such as quantity of connective and subcutaneous tissue. Morphological aspectsof skin should be taken into account in studies that involve optical therapy and diagnosis in murine models.

9531-84, Session PSat

Study of the vitamins A, E and C esters penetration into the skin by confocal Raman spectroscopy

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Liposoluble derivative of the vitamin A - retinyl palmitate, vitamin E - tocopheryl acetate, and vitamin C - tetra-isopalmitoyl ascorbic acid, are frequently used as an active ingredient in cosmetic products.

The aim of this work is to track and compare the absorption of these derivatives and their encapsulated form, into the healthy human skin in vivo using Confocal Raman Spectroscopy.

Significant difference in permeation of the products was observed. Whereas only free form of retinyl palmitate penetrate the skin within first 15 minutes, all 3 vitamin derivatives were present under the skin surface in case of encapsulated form.

9531-85, Session PSat

Grey hair analysis by confocal Raman spectroscopy

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The objective of this study was to apply noninvasive confocal Raman spectroscopy (CRS) to investigate whole virgin grey hair. The results of the analysis of the virgin grey hair depth profiling are spatially correlated to structural organization and chemistry of the hair. It is possible to identify changes in amino acid content, keratin secondary structure, disulfide cross-links distribution, and lipid chains conformation.

These data indicate that SRC could be successfully applied for the whole hair, and could be an effective tool in the evaluation of the efficacy of specific treatment of the hair.

9531-86, Session PSat

Laser phototherapy effects on muscle relaxation prior to articulator mounting in central relation

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Central Relation (CR) obtention often demands prior procedures, such as the use jigs muscle relaxants, due to muscle fatigue. However these procedures increase cost and number of sessions as well as cause patient discomfort. Literature has shown positive clinical effects of laser phototherapy (LPT) on muscle tissues by promoting reducing pain and acute inflammation and also accelerating tissue repair. The objective of this study was to evaluate the influence of LPT in deprogramming muscles for articulator mounting in CR. Patients subjected to oral rehabilitation were submitted to LPT using a continuous diode laser (660nm, InGaAlP, beam area of 0.04cm2), in punctual and contact mode in four points per area: masseter and temporal muscles, and along the mandibular condyles bilaterally. The irradiation parameters were: 40mW, 1W/cm2, 50J/cm2, 50s and 2J per point. Electromyographic (EMG), ultrasound (US), bite strength (BS) and masticatory efficiency (ME) analysis were carried out before and after LPT in mastication muscles and patients were asked to answer a questionnaire. Data from EMG, US, BS and MG and questionnaire analysis demonstrated the ability LPT to immediately promote muscle relaxation and patient comfort. LPT proved to be quite favorable in deprogramming the muscles, wich in turn can promote a more accurate articulator mounting in CR. Therefore, LPT can be helpful in oral rehabilitation optimizing the treatment and promoting patient comfort.

9531-87, Session PSat

Portable widefield imaging device for ICD-detection of the sentinel lymph node

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The sentinel lymph node (SLN) is the first lymph node that drains the cancer cells. When the SLN is positively detected with malignant cells, this indicates cancer regional spreading, and requires further investigation of the potential metastasis to other organs. In this way, the biopsy of the SLN during tumor resection is one relevant diagnostic procedure to further treatment planning. There is no previous knowledge which lymph node in the local system is the SLN, so its location within muscle tissue is still a medical challenge. The injection of the indocyanine green (ICG) at the tumor borders and its fluorescence detection through a widefield imaging device aid the detection of the SLN. A portable imaging device was assembled for the ICG-based detection of the SLN. The device is composed of an array of diode lasers centered at 780 nm, optical components (dichroic mirror and bandpass filters), and two CCD cameras. One camera takes white light image, and the second one the fluorescence emission around 810 nm. A software developed using LabVIEW platform merges the images and provides a real time visualization of the fluorescent emission superimposed in the white light image. The imaging device was first tested in ex vivo muscle tissue, and in an animal model. The first images in a patient of tongue carcinoma resection will also be presented. The results show the potential of a portable and low cost imaging device for the SLN detection during tumor resection.

The system of fluorescence of Indocyanine Green (ICG) is composed of a system capable of illuminating at the wavelength of 780nm. This system has LASER diodes with a homogeneous distribution of light. For detection is used a dichroic mirror which allows the passage and the selective reflection of light for cameras equipped with filters that bus certain wavelengths, selecting the spectral region for the formation of the image. The cameras capture images from the system, being a responsible for acquisition of visible light and the other responsible for the acquisition of fluorescence of ICG. The received images are handled in software developed under the platform LabVIEW® generating an image superimposed end where it is possible to observe the field visible and the region of fluorescence.

Experiments with mice and images in human has confirm the applicability of the product.

9531-88, Session PSat

Evaluation of cotton-fabric bleaching using hydrogen peroxide and blue LED

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The raw cotton production requires multiple steps being one of them the removal of impurities acquired during previous processes. This procedure is widely used by textile industries around the world and is called bleaching. The raw cotton is composed by cellulosic and non-cellulosic materials like waxes, pectins and oils, which are responsible for its characteristic yellowish color. The bleaching process aims to remove the non-cellulosic materials concentration in the fabric increasing its whiteness degree. The most used bleaching method utilizes a bath in an alkali solution of hydrogen peroxide, stabilizers and buffer solutions in high temperature. In the present work we evaluated the possibility of using a blue illumination for the bleaching process. We used blue LEDs (450 nm) to illuminate an acid hydrogen peroxide solution at room temperature. The samples treated by this method were compared with the conventional bleaching process through a colorimetric analysis and by a multiple comparison visual inspection by volunteers. The samples were also studied by a tensile test in order to verify the integrity of the cloth after bleaching. The results of fabric visual inspection and colorimetric analysis showed a small advantage of the sample treated by the standard method. The tensile test showed an increasing on the yield strength of the cloth after bleaching using blue light. The presented method has great applicability potential due to the similar results with the standard method, relative low cost and reduced production of chemical waste.

9531-89, Session PSat

Analysis of the spectral variability of the human skin by in vivo confocal Raman spectroscopy

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The skin is a complex structure, divided into layers, endowed of various biochemical components, which along with other factors provide the equilibrium of the organism. Biochemical composition of the skin changes in each layer and therefore the depth spectral profile very with the depth.

Therefore, the main goal of this study was to determine the spectral variability in different regions and depth of the human skin in vivo. Confocal Raman spectroscopy skin studies were performed in the stratum corneum layer at different arm regions and depth (from the surface down

to 20 ?m), to verify the variation of the concentration of biochemical constituents. This is a complex experiment due to the involuntary movements at the time of the measures, intrinsic variations in different layers of the skin, changes in the movement of the microscope objective. Thus, determination of the variability of these components is of utmost importance, so that the data obtained by the in vivo experiments can be validated.

The Raman spectra were collected from 10 healthy female voluntaries using a confocal Raman system from Rivers Diagnostic, with 785 nm excitation line and a CCD detector. Measurements were performed in the forearm region, at three different points at different depth, with spatial resolution up to 1 ?m. For each depth point, three spectra were acquired. Standard deviation were calculated to evaluate the variability.

It is found that the standard deviation is not significant for the same depth at different positions on the forearm.

9531-90, Session PSat

Histological and biomechanical evaluation of the effect of low level lasers on skin wound healing in rats

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Tendinopathies are musculoskeletal disorders common with a variety of therapies and treatments and the most widely used is the drug for pain relief with satisfactory results due to their undesirable effects with prolonged use.

In this scenario, the search for new therapies unlike the traditional assumes lead role in the medical field. The low level laser appears as promising therapy on modulation of acute and chronic inflammatory process with absence of adverse effects. However, the establishment of clinical parameters for the use of this technique is extremely important for your validation. We investigate the effect of low level laser therapy in acute inflammatory process of the Achilles tendon of rats. 25 wistar rats were used, between 150 g to 200 g. The animals were anesthetized with association of xylazine hydrochloride and ketamine hydrochloride (90 mg/Kg and 10 mg/Kg respectively, intraperitoneal injection). After anesthetized, each animal received transcutaneous injection of collagenase in concentration of 1 mg/ml (Sigma Chemical Co, Cat. C-6885) and 100 μl volume in the posterior region of the right paw in the Achilles tendon. The animals were divided into 5 groups of 5 animals: control (C), tendinitis (T), anti-inflammatory treatment (D), lasertherapy (1J) and lasertherapy (3J). The pharmacological treatments and laser therapy (1J and 3J, 100mW), were carried immediately after the induction of tendinitis. After euthanasia by overdose, 2hours after tendinitis induction, the tendon tissue was removed for analysis of gene expression (MMP3, MMP9 e MMP13) and mechanical properties.

Tendinitis induction model by collagenase injection increases the expression of matrix metalloproteinases (MMP 3, 9 and 13), and decreases some mechanical properties, presented in the Group (NT). The anti-inflammatory was unable to reduce the inflammatory process by expression of MMPs in charge of tendon tissue degeneration.

The low level laser (3J) appears to modulate the inflammatory process, reducing the expression of MMPs and improving the mechanical properties of the tendon. Suggests that the low-level laser is able to reduce the tissue degeneration, leading to a better quality of biomechanics in the tendon.

9531-91, Session PSat

The importance of technological advance in the search for quality in health care

Rosely Cordon, CEPI FOUSP (Brazil)

Attention to quality in health care, is the type of attention that maximizes the well being of the patient, after taking into consideration the results of expected gains, and contemplating the process in all of its parts, it is the degree to which the characteristics of a product/service fulfill the objectives for which it was created, as such, in the area of health care, the lack of quality means having risks. Quality is consciencousness and initiative, Adaptation to use , Conformity to specification , Client satisfaction . Growth and innovation : it is the capacity of an organization to maintain an infrastructure of people, equipment and technology that support its development over the long term. To adequately incorporate a technology, in this case the use of laser in odontology, we have to have Strategies that guarantee quality. The evaluation of a technology tends to be incorporated by the health care system, public or private, characterized by systematic review, critical and criterius, of available literature, considering aspects such as effectiveness of the intervention, economic analysis of it, and its potential impact on the health care system, in other words, its contribution towards promoting, maintaining or recuperating health.

INCORPORATING NEW TECHNOLOGIES IS LINKED TO: New technologies are associated with an increase in costs, but not always with proof of benefits to the user. Cumulative incorporation: New and old technologies work together, Influence medical practices, Stimulated by health care professional, User is an easy captive of health care marketing (direct and indirect), The offer influences the demand. NEW TECHNOLOGIES WHEN WELL USED THEY BRING BENEFITS TO EVERYONE, SUCH AS: Greater client satisfaction, personal involvement in the prevention and/or solution of problems, better documental control, better traceability, homogeneity and stability of the process, error reduction/ reworking reduction, waste reduction/ cost reduction, establishment and implementation of joint measures, monitoring by means of indicators, easy access to information, national and international recognition of the quality of the process, more objective and effective training, improvement in programs for balancing and maintenance of equipment.

Coclusion: Care with strategic management, such as: infra-structure, adequate documentation, precautions with standards and occupational risks, flow and process, and with the divulging of scientific articles based on good research; we have a technology, in the specific case of this project, the use of laser in odontology, that is multi-disciplinary, contributing to health care.

9531-92, Session PSat

Effects of low level laser in the morphology of the skeletal muscle fiber during compensatory hypertrophy in plantar muscle of rats

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Introduction: Skeletal muscle is composed of fibers having a high plasticity and capable of remodeling in response to various stimuli. The hypertrophy is known as an increase the cross-sectional area of the muscle as a result of a muscular work against an overload, and is compensatory because the overload is induced by functional elimination of synergistic muscles.The importance of study the compensatory hypertrophy is understand how this process can be influenced by the irradiation with regard to the weight and muscle cross-sectional area, to assist in the rehabilitation process and the effectiveness functional return. Objective: The aims of present paper was the evaluate the effects of low level laser irradiation on morphological aspects of muscle tissue, comparing the weight and cross-sectional area in rat skeletal muscle with compensatory hypertrophy. Methods: Wistar rats weighing 242,5g ± 13,59 were divided into three groups: control (n=5) (without plant ablation and without irradiation), hypertrophy group without irradiation (n=10) (right

plantar muscle) and hypertrophy group and irradiation (n=10) (irradiation in the left plantar muscle), both analyzed after 7 and 14 days. The ablation model consisted of partial removal of synergist muscles causing overload of plantaris muscle.The irradiation was performed daily immediately after the surgery with an AlGaAs. The parameters were: ? = 780nm, beam spot of 0.04cm2,output power of 40mW,power density of 1W/cm2, energy density of 10J / cm2 and 10s exposure time with a total energy of 3.2 J. The animals were euthanized at 7 and 14 days. The plantar muscles were carefully removed and weighed on a precision balance with three decimal places. Morphological aspects were evaluated using H&E staining. The circumference of each fiber marked using ImageJ to generate cross - sectional area was obtained.

Results: The results revealed that low level laser irradiation an increase the weight of the plantaris muscle after 7 and 14 days after bilateral removal of the synergist muscles with a difference of 7.06% and 11.51% respectively. There was a significant increase between the cross-sectional area of the group with compensatory hypertrophy (irradiated and non-irradiated) as compared to control at 7 and 14 days and there was a significant increase in cross-sectional area in the irradiated group compared to non-irradiated group within 14 days (* p <0.001).

Conclusion: In conclusion, low level laser irradiation has an effect on compensatory hypertrophy to produce increased muscle weight. It was possible to conclude that LLL irradiation, wavelength 780 nm and parameters cited above, promoted an increase in cross-sectional area of muscle fibers in the compensatory hypertrophy model after 14 days.

9531-93, Session PSat

Optimization of parameters for photoinactivation of E. faecalis using factorial design

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Aiming to solve the increased bacterial drug resistance the photodynamic therapy appears as an alternative treatment for microbial photoinactivation. Photodynamic Inactivation (PDI) is based on oxidative destruction of biological molecules through production of reactive oxygen species generated by photo-excitation of the photosensitive molecule. Hypericin presents a high quantum yield of the triplet state and singlet formation besides not being toxic in the dark, being potentially increased its action in the presence of light. In order to determine the best parameters to minimize the survival index of E. faecalis, a 23 factorial design was used to define the parameters as light dose (3-6 J.cm-2) using a Biotable with emission at 590±11 nm, hypericin in concentration (0.0008-0.075 μg/ml) and incubation time (2-4 min). The Software Action and Statistica 8.0 was used to calculate the effects and interactions between the parameters with a confidence interval of 95%. The Pareto diagram showed that the accumulation kinetics of hypericin is fast, and the incubation time is not a significant parameter (p <0.05) as well as the interaction between the three factors and the interaction between time incubation with concentration and incubation time with dose. However, light dose with concentration as well as their interaction are significant

parameters and inversely proportional to the survival index (p>0.05). The response surface methodology (RSM) showed that the best results of inactivation are obtained with at concentration 75 ng/ml, light dose 3,9 J.cm-2 and incubation time of 2 minutes.

9531-94, Session PSat

Kinetics of photobleaching of methylene blue in collagen matrix measured with CCD camera

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The photobleaching (PhB) is a photo-induced degradation of photosensitizer, with its decreasing concentration and consequently the ability to absorb photons, leading to reducing its ability to formation of reactive species. If the PhB happen before cells are affected, will not damage occurs to them, it is suitable for therapy exposed healthy cells and not suitable for treating malignant cells. Therefore, the PhB is related to efficiency of Photodynamic Therapy (PDT). Dynamic images of samples containing 100 ?M methylene blue and 240 mg of hydrolyzed collagen in milli-Q water (10 mm fluorescence cuvette), were recorded with a standard CCD camera (10 frames/sec) during the irradiation with a 635nm diode laser (Coherent-USA) of initial power equal to 7mW. Our previous results show that the PhB occurs in 2-steps: a faster step (few seconds), with an intense penetration of light (~40% of deep penetration); followed by a slower penetration of light, with monoexponential decay rate. The results show that after 240 seconds photobleaching already happened longitudinally through the sample, with time rate ~9.7?10-3 s-1. The photobleaching of MB is associated to the aggregate state of the dye, a condition in which free radicals are generated while the monomeric form does not bleach under irradiation. Therefore, when immobilized in collagen matrix the MB molecules probably are caged in the matrix in the aggregated and in the monomeric forms. The photobleaching continues to grow axially after 240 seconds. We are working to calculate the kinetic rates of axial photobleaching through the samples.

9531-95, Session PSat

Study of limitation in visualizing dental root canals of bovine specimens imaged by dental transillumination

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In recent years our research group is studying how detect dental pulp vitality by transilluminating near infrared radiation (NIR) through teeth. First, we prove viability in visualizing pulp chamber of human incisive and premolar teeth with transillumination even dentin being a high scattering tissue. This article gives sequence to study presenting initial results of root canals tranilluminated with NIR. Here we are interested to study how optical properties of dentin could limit the visualization of root canals varying optical intensity of illumination, sensor's exposure and sample thickness. For experimentation, we composed six groups of bovine dental root with three samples each. We sanded the vestibular and lingual surfaces of samples in order to reach desired thickness. Then, it was composed experimental groups with 1,0mm; 2,0mm; 3,0mm; 4,0mm, 5,0mm and 6,0mm thickness. For imaging a FPA InGaAs camera model Xeva 1.7-320 (900nm-1700nm; Xenics, Inc., Belgium) and a 3W lamp-based broadband light source (Ocean Optics, Inc., USA) were used. This camera still allows us imaging sample controlling the sensor's time of exposure in 1ms or 20ms. Bandpass optical filters at 1000nm±10nm, 1100nm±10nm, 1200nm±10nm and 1300nm±50nm spectral region completed the imaging system; we also captured images without using the optical filters (broadband imaging). Images resulted from experiment reveal root canals of all specimens with thickness up to 3,0mm when put the light source in contact with samples and without using the filters. Above that thickness threshold, scattered radiation blurred the visualization of canals and it is necessary a processing on images. Results are not dependent with the time of exposure of FPA sensor.

9531-96, Session PSat

Confocal Raman study of aging process in diabetes mellitus human voluntaries

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The phenomenon of glycation reaction in which a reducing sugar binds to a protein without the presence of an enzyme originates AGEs [Advanced Glycation End –products], formed by a heterogeneous class of molecules, which are crosslinked with proteins. |Accumulation of AGEs occurs slowly during the process of aging of the human skin in situations of diabetes mellitus and its pronounced formation is accelerated. In this work, we perform the analysis of human skin in diabetes mellitus voluntaries by confocal Raman spectroscopy. This technique uses a laser of 785 nm, as excitation source, which focuses on the skin through a microscope objective lens and the inelastic scattering light, which gives information of biochemical composition, was collected by a CCD detector. Our aim was to characterize the aging process resulting from the glycation process volunteers, in a separate group of 10 healthy young women, 20-30 years old and 10 healthy women , 56-81 years and 10 older women and women with type I and II diabetes, 56 to 81. The spectra were analyzed in the finger print region of 400-1800 cm-1 in the dermis at a depth of 70-130 mícrons. A molecular interpretation of changes in type I collagen in the dermis was performed through the screening of the bands of AGEs also by changes in the intensities of the peaks of proline and hydroxyproline in women with diabetes compared to healthy elderly and young, thereby enabling concluding that the aging caused by glycation of proteins process degrades type I collagen differently and leads to accelerated aging.

9531-97, Session PSat

Effect of laserphototherapy on human alveolar bone repair: microtomographic and histomorphometrical analysis

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The immediate dental implant placement in the molars region is critical, because of the high amount of bone loss and the discrepancy between the alveolar crest thickness and the dental implant platform. Laserphototherapy (LPT) improves bone repair thus could accelerate the implant placement. Twenty patients were selected for the study. Ten patients were submitted to LPT with GaAlAs diode laser (808nm) during molar extraction, immediately after, 24h, 48h, 72h, 96h and 7 days. The irradiations were applied in contact and punctual mode (100mW, 0.04cm2, 0.75J/cm2, 30s per point, 3J per point). The control group (n=10) received the same treatment; however with the power of the laser off. Forty days later samples of the tissue formed inside the sockets were obtained for further microtomography (microCTs) and histomorphometry analyses. Data of both groups were compared by the Student t test, whereas those from the different microCT parameters were compared by the Pearson correlation test ($p<0.05$). The relative bone volume, as well as area were significantly higher ($p<0.001$) in the lased than the control group. In the control group there were negative correlations between number and thickness, and between number and separation of trabecula ($p<0.01$). Between thickness and separation of trabecula the correlation was positive ($p<0.01$). The laser group showed significant negative correlation between the number and the thickness of trabecula ($p<0.01$). Laserphototherapy accelerated bone repair. By the Pearson correlation test it was possible to infer that the lased group presented a more homogeneous trabecular configuration, which would allow earlier dental implant placement.

9531-98, Session PSat

Multifunctional luminomagnetic bioimaging contrast agents for medical imaging

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Contrast agents with multiple functionalities have several importance in the medical imaging community. Current research has proposed the use of various materials with duel functionality which are normally restricted in a single modality such as fluorophors, dyes, and quantum dots. In this project, we are proposing the development of NIR based nanocrystals (NCs) with multimodal features for the potential use in medical imaging. The multimodal imaging comprises optical imaging, magnetic imaging, and X-ray imaging by utilizing the superparamagnetic features of Gd3+, the high X-ray excitation cross section of Lu3+, and the NIR fluorescence of Nd3+. Halides such as MGdLuF4 (M=K,Na) were doped with NIR active rare earth ions, Nd3+, where synthesis conditions have been optimized for obtaining the brightest phosphor with a size of < 50 nm. Characterizations of the NPs were done to explore the excitation and emission properties, crystal structure, morphology, magnetization and X-ray excitation properties. The nanophosphors were then coated with poly(maleic anhydride- alt-1-octadicene) (PMAO) to implement in confocal cellular imaging and cellular toxicity to determine the viability and cytotoxicity at different concentrations. Future work for the application of these nanophosphors in medical imaging will be confirmed by magnetic resonance (MRI) as well as positron emission tomography (PET).

9531-99, Session PSat

Optimization of chlorin e6 activation utilizing upconversion energy transfer

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Current cancer therapy techniques possess several drawbacks such as severe side effects and lack of selectivity. Chemotherapy and radiation therapy are the most common treatments, but fail to distinguish between cancer and healthy cells resulting in harmful side effects. Photodynamic therapy (PDT) is one of the fastest emerging techniques due to its many advantages, including the use of nonionizing radiation, targeted delivery, and controlled release of photoactive drugs. In PDT, photosensitizers (PS) are activated inside targeted cells to produce irreversible damage inducing cell death. Since most PS operate in the visible range, it is difficult to activate them due to the high attenuation of soft tissue. Upconverting nanoparticles (UCNP) are able to absorb in the near infrared region, where light is less attenuated, and emit in the visible range, resulting in deeper tissue penetration. UCNPs are able to assist with the activation of the PS by energy transfer when the PS is conjugated onto the UCNP. Chlorin e6 (Ce6) is one of the most used PS due to its ability to release reactive oxygen species (ROS), which is one of the main processes utilized in PDT. The UCNP used contain a combination of rare earth doped ions including Erbium, Thulium, and Holmium precisely doped into the host nanocrystal to improve upconversion emission and energy transfer. The work presented will focus on exploring the factors that affect the activation of Ce6. Furthermore, the results will include the enhancement of Ce6 activation and ROS release when conjugated onto a rare earth-doped UCNP.

9531-100, Session PSat

The effect of phototherapy after a single application for the treatment of temporomandibular joint dysfunction associated with fibromyalgia

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Introduction: The clinical presentation of patients with FM and TMD deserves attention in the somatic pain results in fatigue, headache, morning body stiffness, paresthesia, cognitive impairment and sleep disturbance humor.1,2 Rationale: The clinical complexity of the patient creates the need for treatment modalities that substantially reduce chronic pain. Objective: This study aims to determine the effect of phototherapy in a single dose, and thus to observe the differences when these patients undergo physical exertion. Method: The study protocol was approved by the research ethics committee of the Universidade Nove July (protocol number: 419 828), in which all participants signed an informed consent. All participants had a confirmed diagnosis of FM and TMD, which were performed according to the American College of Rheumatology and the Research Diagnostic Criteria for Temporomandibular Disorders (Axis I: physical; shaft: psychological II), respectively. Were 60 participants were female, the mean age was 45 ± 1.2, BMI 27 ± 2.9, mean height 1.57 ± 3.1. Was randomly divided into three groups: Group Phototherapy (FT, n = 20), Group + Phototherapy Physical Activity (FT + A, n = 20), placebo group (Pl, n = 20). Was carried out only one application of phototherapy on sore areas and physical activity was described as the American College of Reumatologia5, and added 10 minutes of exercises for the face. Data were evaluated with digital algometry. Results: Before the session had the most facial expression and your reports of a lot of pain, depression and discouragement. Soon after phototherapy session in FT group, there was a social relationship closer to most of the volunteers, which resulted in pain as assessed by algometry, a significant improvement in points mainly from the region of the trunk, face and upper limbs, with respect to the body and left hemi regarding the right hemi body, the results are more evenly, but with determinants in the trunk and also factors in the face. Conclusion: This study showed an immediate effect of action of phototherapy on pain in fibromyalgia and TMD independent of exposure to physical stress in these patients.

9531-101, Session PSat

Biochemical differentiation between the trypanosoma cruzi and leishmania amazonensis by FT - IR

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The L. amazonensis specie causes cutaneous Leishmaniasis. While Chagas disease is caused by T. cruzi, which has similar morphology of the Leishmania specie. Indeed, these parasites have phylogenetic similarity because it belongs to the same family and it is quite common the cross-reactions among them in the clinical serological tests. The aim of this study was to use Fourier Transform Infrared (FT-IR) Spectroscopy to characterize and discriminate these parasites. The cultures of T. cruzi and Leishmania were grown in the LIT and 199 media respectively, and the subcultures were held weekly. All cultures were washed with NaCl solution, centrifuged, hydrophilified and characterized by FT-IR. In the characterization of the species was observed changes chemical composition along the infrared spectrum. The statistics results showed separation among the species by dendrogram using Ward´s algorithm. The spectra deconvolution showed a quantitative difference in the vibrational regions of polysaccharides, fatty acids and nucleic acids among the species.

9531-102, Session PSat

Laserphototherapy effect on the activity of alkaline phosphatase of osteoblasts under different concentrations of alendronate and zoledronic acid

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Sodium alendronate and zoledronic acid in different concentrations are bisphosphonates (BPs) currently applied for treating of bone metabolism diseases. These drugs and other antiresorptives and antiangiogenic drugs present as a side effect necrosis of jawbones, known as Medication related osteonecrosis of jaw (MRONJ). Laser phototherapy (LPT) using red wavelength has shown positive effects on MRONJ lesions healing. This study aimed to evaluate the activity of alkaline phosphatase in osteoblasts submitted to sodium alendronate and zoledronic acid associated or not to LPT. Cells were cultured in DMEM containing one of the BPs in different concentrations for 24 hours. The LPT was then applied by using a continuous diode laser (InGaAlP, 660nm, 30mW, spot size of 0.028 cm2) in two different energy densities (5J/cm2 and 10J/cm2, 4,5 and 9s, respectively), on punctual and contact mode. Two irradiations with 6 hours-interval were performed. The activity of alkaline phosphatase 7 days after the first irradiation was evaluated. The zoledronic acid, irrespective to the concentration applied, led to significant decrease in ALP activity. LPT was not able to compensate this inhibition. Sodium alendronate also cause decrease in ALP activity, except when applied at the concentration of 10um in cultures irradiated or not with 5J/cm2. LPT using 10J/cm2 inhibited the activity of this enzyme, for both BPs analyzed. In the parameters here applied the LPT has no effect on the activity of alkaline phosphatase of cells treated with sodium alendronate or zoledronic acid.

9531-103, Session PSat

Influence of 660nm diode laser irradiation on human stem cells of deciduous dental pulp (SHEDs)

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Tissue engineering is based on three elements: stem cells, scaffolds and growth factors. Laserphototherapy (LPT) could be the fourth element in tissue engineering. The aim of this study was to analyze the behavior of dental pulp stem cells from human exfoliated deciduous tooth (SHEDs) submitted to 660nm diode laser irradiation. The proliferation, as well as the stem cell markers gene expression, was studied before and after LPT. The gene expressions of embryonic (Oct-4) and mesenchymal stem cell markers (Nestin, CD90 and CD105) were assessed by real time PCR assay. The cell proliferation patterns of cells under nutritional deficit were evaluated through the MTT reduction assay in 24, 48 and 72 h and. The LPT was carried out with an InGaAlP laser in the following parameters: 660nm, 20mW, spot area of 0.028cm2, two-irradiations of 6h-interval in contact mode, one point, and the energy densities of 5 or 20J/cm2. The data were statistically compared ANOVA and complemented by Tukey´s test ($p \leq 0.05$). Cultures treated with LPT at 5J/cm2 presented significant growth increase along with overexpression of Nestin and CD90 genes. For Oct-4 and CD105 genes LPT had no effect in any of the parameters tested. SHEDs respond positively to LPT in particular irradiation parameters; allowing the cells to restore their proliferative capacity without losing their undifferentiating status. Thus, this therapy could be of importance for improving tissue engineering procedures.

9531-104, Session PSat

Application of time-resolved, intensified camera for fluorescence lifetime imaging of small animals

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We present time-resolved system for small animals fluorescent imaging. The setup is based on a time-gated, intensified CCD camera with fluorescent filters, a supercontinuum pulsed laser with an optical parametric oscillator and an optomechanical switch. The camera collects images of fluorescent photons penetrating a tissue. The images are acquired at different delays in respect to the laser pulse to collect a distribution of time of flight of fluorescent photons. We carried out first experiments on custom-made rat-phantom with indocyanine green inclusions and first experiment on a rat. The distribution of lifetime of fluorescent dye is calculated with a custom-made software. The expected distribution of time of flight of fluorescent photons is calculated from deconvolution of measured signal and instrument response function. Furthermore the lifetime is calculated based on fitting an exponential function. In the next step we plan to carry out phantom experiments with the use of dyes with long, single microsecond, lifetime and then continue experiments on small animals.

9531-105, Session PSat

Band deconvolution analysis of infrared spectra of the saliva for monitoring physiological stress in athletes

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The monitoring of physiological stress in athletes has been done by analysis of salivary biomarker. However, the clinical trials to these biomarkers are very expensive that is not accessible for all sports teams. In this study, the athlete's saliva (n=13) was analyzed by Fourier Transform Infrared (FT-IR) spectroscopy, which was used to identify changes in the spectral regions assigned to cortisol (stress biomarker). Saliva samples were collected before (B), after physical effort (A) and after 2h recovery (R). A drop of sample (15 μl) was deposited on calcium fluoride window and dehydrated for 30 min. Spectra were recorded in the spectral region between 4000 to 750 cm-1 (32 scans and resolution of 4 cm-1). The band deconvolution analysis was done in the spectral region assigned to cortisol at 1180 - 955 cm-1. The calculated values of the bands area at 1121 cm-1 and 1079 cm-1 were, respectively: B) 12.01 and 77.18; A) 5.07 and 84.39; and R: 10.32 and 76.51. The results show that R values are approximately the same of B values, which has similar response using laboratory techniques. The advance of FT-IR was the small volumes of samples required and there is no need of chemical reagents.

9531-106, Session PSat

Analysis of membrane negative electrical charges and the bond profile of the mannose binding lectin in sickle cells using cationic quantum dots

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Sickle cell anemia is the most frequent hereditary hemoglobinopathy worldwide. Sickle (HbSS) erythrocytes are subject to modifications when exposed to a low oxygen concentration, which can include changes on their membrane lipids distribution and the decrease of the HbSS cell elasticity. These biophysical modifications can also reduce negative charges of HbSS membranes, which lead to the decrease of the Zeta Potential, a property that prevents erythrocytes of agglutinating, not only among themselves, but also with endothelial cells. More recently, it has been investigated that these HbSS modifications can expose membrane receptors allowing the bond of the MBL (mannose-binding lectin). The MBL is a protein that can help the removal of sickle erythrocytes from vessels and prevent the vase-occlusive crisis in sickle cell anemia patients. In this work, cationic quantum dots (QDs) stabilized with cysteamine, enabled, by their electrostatic interactions with negative erythrocyte membranes, not only the analysis of changes in electric charges of HbSS surfaces, compared to normal ones (HbA), but also the investigation of the MBL binding to those cells. According to flow cytometry analysis, revealed by the QDs labeling pattern, HbSS cells showed a decrease of their membrane charges in about 10%, when compared to HbA. Fluorescence microscopy images confirm these results. Furthermore, these analyses also displayed that the MBL binds to membranes of both HbA and HbSS erythrocytes, preventing the labeling by QDs. These outcomes show that cationic QDs can be a valuable tool for elucidating cellular alterations essential to the comprehension of the physiological changes resulting from sickle cell anemia or other diseases.

9531-107, Session PSat

Shrinkage of porcine cutaneous specimen after formalin fixation and histopathology preparation: utilising OCT for dimensional change measurements

Dara B. Rashed, Eastman Dental Institute (United Kingdom)

A porcine model was utilized in this study in order to evaluate and measure the percentages of both horizontal and vertical dimensional changes (contraction or shrinkage and expansion), using a new in-situ OCT oral instrument as well as the standard OCT dermatology instrument, before formalin fixation (fresh or dry porcine skin), after seven days fixation of the porcine model in 10% NBF (Neutral Buffered Formalin), as fixation for a short period of time (between 24-48 hours) lead to incomplete processing, thus the duration of fixation can affect the degree of tissue shrinkage. In addition, we have also measured the dimensional changes for the porcine skin histopathology slides.

Results show that the mean percentage of tissue expansion and shrinkage in horizontal (X) plane as a result of formalin fixation was about 7.22% for OCT skin instrument, while for OCT oral instrument was about 7.40%, whereas in vertical (Z) plane the mean percentage of tissue shrinkage was 7.01% and 7.44% for OCT skin and oral instruments respectively. While the mean percentage of tissue expansion and shrinkage in horizontal (X) plane for the histopathology sections/images was greater compared to formalin fixation of the porcine skin tissue and was about 11.33% for OCT skin system, while for OCT oral system was about 11.23%, whereas in vertical (Z) plane the mean percentage of tissue shrinkage was 12.45% and 12.82% for OCT skin and oral systems respectively.

9531-108, Session PSat

Evaluation of effects of refractive index change on new OCT oral instrument using different porcine tissue models

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A fresh porcine skin with the underlying subcutaneous fat and muscle were utilized in this study with a number of scalpel blades (Surgical Scalpel Blade, No. 15, Swann-Morton, UK) penetrating the tissue models at 40-60° angle. The scalpel blades were used to penetrate the samples so as to evaluate the effect of refractive index (RI) change on the OCT laser light beam refraction by observing apparent image distortion as a result of differing indices of refraction of the overlying scanned media, i.e. skin, adipose tissue, and muscle samples, where each sample scanned separately. We used a recently modified and validated OCT oral instrument as well as the standard OCT dermatology instrument versions 2.1 and 2.0, respectively (VivoSight® Michelson Diagnostics Ltd, Orpington, Kent, UK) to scan the fresh samples, then re-scanning the samples after being immersed for three days in phosphate buffered saline. Following this, the tissue samples were scanned once more by both OCT systems after being frozen for three days.

Results illustrated that the modified non-CE certified OCT oral instrument is performing similarly to the standard CE certified OCT skin instruments and offered comparable and trustworthy images when compared to OCT skin system images. Consequently, we suggest that the OCT oral system can be used as direct surrogate for the standard OCT skin instrument in measurement of cutaneous and oral tumour thickness and depth with confidence and accuracy.

9531-109, Session PSat

Characterization of caries progression on dentin after irradiation with Nd:YAG laser by FTIR spectroscopy and fluorescence imaging

Patricia A. Ana, Adrianne M. M. Brito D.D.S., Univ. Federal do ABC (Brazil); Denise M. Zezell, Instituto de Pesquisas Energéticas e Nucleares (Brazil); Emery C. Lins, Univ. Federal do ABC (Brazil)

Considering the use of high intensity lasers for preventing dental caries, this blind in vitro study evaluated the compositional and fluorescence effects promoted by Nd:YAG laser (?=1064 nm) when applied for prevention of progression of dentin caries, in association or not with topical application of acidulated phosphate fluoride (APF). Sixty bovine root dentin slabs were prepared and demineralized by 32h in order to create early caries lesions. After, the slabs were distributed into six experimental groups: G1- untreated and not submitted to a pH-cycling model; G2- untreated and submitted to a pH-cycling model; G3- acidulated phosphate fluoride application (APF); G4- Nd:YAG irradiation (84.9 J/cm2, 60 mJ/pulse); G5- treated with Nd:YAG+APF; G6- treated with APF+Nd:YAG. After treatments, the samples of groups G2 to G6 were submitted to a 4-day pH-cycling model in order to simulate the progression of early caries lesions. All samples were characterized by the micro-attenuated total reflection technique of Fourier transformed infrared spectroscopy (μATR-FTIR), using a diamond crystal, and by a fluorescence imaging system (FIS), in which it was used an illuminating system at ?= 405±30 nm. Demineralization promoted reduction in carbonate and phosphate contents, exposing the organic matter; as well, it was observed a significant reduction of fluorescence intensity. Nd:YAG laser promoted additional chemical changes, and increased the fluorescence intensity even with the development of caries lesions. It was concluded that the compositional changes promoted by Nd:YAG, when associated to APF, are responsible to the reduction of demineralization progression observed on root dentin.

9531-110, Session PSat

Fiber spectroscopy for cancer diagnostics in-vivo

Viacheslav Artyushenko, art photonics GmbH (Germany)

Review of fiber spectroscopy systems using all main spectroscopy methods in the range from 200nm to 16μm: fluorescence, diffused reflection, Mid IR-absorption and Raman scattering, - with the main focus on fiber probes design used in two areas:

1) In labs – for in-line monitoring of Multi-Organ Chips (MOC) and for tissue analysis in-vitro;

2) In clinics – realized with tiny probes for endoscopic or laparoscopic applications, including:

a) ATR-fiber probes made from innovative Polycrystalline IR-fibers (PIR-fibers), and

b) fluorescence, Near IR and Raman probes made from silica glass fibers.

The latest development in Mid IR-fiber optics extends the spectral range covered by biospectroscopical methods from UV-Vis-Near IR to Mid IR to 18μm (20.000 to 550cm-1). Up to now, all fiber systems configured for biomedical diagnostic applications using absorption/transmission, reflection, fluorescence and Raman-spectroscopy methods – were restricted to silica fibers with a transmission from 180 nm to 2.4 μm. Nowadays, IR-glass fibers, Polycrystalline PIR-fibers and Hollow Waveguides can also cover the Mid IR-range up to 18μm, including the "finger-print" region where specific absorption bands of molecular vibrations are concentrated. These fundamental vibrational bands in the MIR are 100-1000 times more intensive and defined compared to their 2nd & 3rd overtones found at shorter wavelengths (<2μm). Spectroscopy using ATR-probes for the analysis of tissue (with sensitive tips using Attenuated Total Reflection) is based on tissue absorption analysis in the range from 2 to 16μm.

Results obtained by ATR-absorption spectroscopy will be compared to Raman spectroscopy - as the both methods compliments each other in molecular vibration analysis. In difference with high tissue absorption in Mid IR typical laser wavelength used for Raman scattering excitation can propagate in tissue much deeper - providing the synergy advantage from these complimentary method combination in more specific and accurate cancer diagnostics..

9531-111, Session PSat

Application of quantum dots in the diagnosis of neglected diseases

Kilmara H. G. Carvalho, Aluizio G. Brasil Júnior, Univ. do Estado de Amazonas (Brazil); Cynarha D. C. Silva, Andresa P. Oliveira, Valeria Pereira, Maria Edileuza F. Brito, Fundação Oswaldo Cruz (Brazil); Regina Celia B. Q. Figueiredo, Adriana Fontes, Beate S. Santos, Univ. Federal de Pernambuco (Brazil)

The present study focuses on the application of semiconductor quantum dots (QDs) as alternative fluorescent probe in immunocytochemical methods for serological diagnostic of neglected diseases. We tested the detection of American Tegumentary Leishmaniasis (ATL), caused by the protozoa Leishmania. L. (V.) braziliensis specie, which is the main etiologic agent of leishmaniasis in Brazil. This disease is endemic and still presents difficulties in its diagnosis. Fluorescent semiconductor nanoparticles of CdTe QDs (~3 nm) stabilized with mercaptosuccinic acid were coupled covalently to anti-human Immunoglobulin G (Anti-IgG) by applying zero dimension coupling agents. A semiquantitative technique using a fluorescence plate reader was employed to monitor the bioconjugation effectiveness. The human QD-Anti-IgG bioconjugates were tested for the detection of human antibodies for the L (V.) braziliensis promastigotes by indirect immunofluorescence (IIF). Our preliminary confocal data analysis (Leica SPII-AOBS) demonstrated the capacity of the CdTe-Anti-IgG bioconjugates to bind to immunoglobulin G directed against ATL in the positive human serum for ATL by IIF, but not in serum from normal

healthy individuals. The overall results show that QDs bioconjugates can be applied as new tools in the development of diagnosis method not only for LTA but also to other neglected diseases.

9531-112, Session PSat

Automatic analysis of microscopic images of RBC aggregation

Pablo Menichini, Univ. Nacional de Rosario (Argentina); Mónica Larese, CIFASIS (Argentina); Bibiana D. Riquelme, Univ. Nacional de Rosario (Argentina)

Red blood cells (RBCs) aggregation is one of the most important factors in blood viscosity at stasis or at very low rates of flow. The basic structure of this aggregations is a linear array of RBCs, arranged like a stack of coins, and commonly termed as rouleaux; interacting rouleaux can form more complicated three-dimensional structures termed amas. In plasma, RBC aggregation is due to the presence of large proteins (e.g., fibrinogen, macroglobulins) and aggregation is enhanced when their level is elevated. Hence, enhanced or abnormal aggregation is seen in clinical conditions, such as diabetes and hypertension, producing various alterations in the microcirculation, some of which can be analyzed through the characterization of aggregated cells. In these diseases, the shape of rouleauxs is altered, forming large clusters of globular shape, which hinder the microcirculation.

So far, image processing and analysis for the characterization of RBC aggregation is done manually or semi-automatically using interactive tools.

Because it is interesting to perform the adaptation as a routine used in hemorheological and Clinical Biochemistry Laboratories, it is important to find an automatic method that is rapid, efficient and economical , and at the same time independent of the user performing the analysis (repeatability of the analysis). We propose a system that processes images of RBC aggregation and automatically obtains the characterization and quantification of the different types of RBC aggregates.

9531-113, Session PSat

In vitro analysis of erosion progression in dentine lesions after laser irradiation of Nd: YAG and fluoride using optical coherence tomography (OCT)

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This study aimed to evaluate by Optical Coherence Tomography (OCT) the progression of lesions in dentin erosion in vitro after irradiation with Nd: YAG laser and topical fluoride in 120 samples of bovine dentin. The samples were protected with acid resistant varnish, with the exception of the central area of 2 mm diameter and divided into 8 groups (n = 15) and subjected to acidic cycling with citric acid solution for 20 minutes, 2 times day, for 20 days. After 10 day acid challenges, each group received a different treatment: a control group (no treatment), one fluorine group (topical sodium fluoride 2% -by 4 minutes); three groups Laser Nd: YAG laser irradiation on contact (1 W, 0.7 W and 0.5 W); and three treated groups associating the fluoride to laser irradiation. The OCT readings were at day 1 prior to first acid challenge (OCT1); 05 (OCT2); 10th (OCT3); Day 15 (OCT4); 17 (OCT5) and day 20 (OCT6). OCT images enabled to evaluate the amount of tooth substance lost over time before and after treatment, and monitoring the progression of early demineralization. After statistical analysis, the fluoride group showed the smallest loss of mineralized tissue. The OCT technique has shown promise for the diagnosis and monitoring of erosion damage, but more research is necessary to adapt its , both in vitro and in vivo.

9531-114, Session PSat

Photophysics of tetracarboxy-phthalocyanines and evaluation of the photodynamic efficacy in model systems

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In the last years many efforts have been employed aiming the searching for efficient photosensitizers for applications in Photodynamic Therapy (PDT). With this purpose, phthalocyanine derivatives has been shown promising compounds due to their high molar extinction coefficient in the 600 - 800 nm region and their high singlet oxygen quantum yields under irradiation. The main functions and mechanisms of photodynamic action for phthalocyanine derivatives are based on their photophysical characteristics, which are governed by their physico-chemical properties. It is well established that modifications in the environment or on its molecular structure can change its optical and photophysical features. In this way, various strategies to obtain phthalocyanine derivatives with better solubility in aqueous media or improved pharmacokinetic features have been reported. These methods primarily involve the incorporation of outlying groups in the phthalocyanine ring to diminish the aggregation or of metal atoms to increase the triplet formation. In this work the effects of substituents and solvents on the photophysical and photochemical parameters of tetracarboxy-phthalocyanines are reported. The complexes studied were the zinc and aluminium tetracarboxy-phthalocyanines in dimethyl sulfoxide (DMSO) and in phosphate buffered saline (PBS) environments. Besides, the present work provides a comparative study between these three photosensitizers evaluating the photo-degradation of proteins (BSA), the time for the photosensitizers to cause photo-hemolysis of red blood cells as well as the relative lipophilicity of the compounds. It was found that the presence of metal atoms changes their photophysical properties and reduces the time of photo-hemolysis and of photo-degradation of proteins.

9531-115, Session PSat

Thermographic diagnostics to discriminate skin lesions: a clinical study

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Cancer is responsible for about 13% of all causes of death in the world, over 7 million people die annually of this disease. In most cases, the survival rates are greater when diagnosed in early stages.1 It is known that tumor lesions present a higher temperature compared with the normal tissues, due to an increased vascularization. Some research has been developed in an attempt to establish new diagnosis methods, targeting this temperature difference. 1,2 In this study, we aim to investigate the use of a handheld thermographic camera to discriminate skin lesions. The patients with Basal Cell Carcinoma, Squamous Cell Carcinoma, Actinic Keratose, Pigmented Seborrheic Keratose, Melanoma, Dysplastic Nevus or Intradermal Nevus lesions have been investigated at the Skin Departament of Amaral Carvalho Hospital. Patients are selected by a dermatologist, and the lesion images are recorded using a infrared camera (Fluke® model FLK-Ti400). The images are evaluated taken into account the temperature level, and differences into lesion areas, borders, and between altered and normal skin. The present results show that thermography may be an important tool for aiding in the clinical diagnostics of superficial skin lesions.

9531-42, Session PIn2

Plasmonic nanosensors and nanoprobes: harnessing the power of photonics for medical diagnostics and therapy

Tuan Vo-Dinh, Fitzpatrick Institute for Photonics, Duke Univ. (United States)

This lecture provides an overview of recent developments in our laboratory for several plasmonic nanoplatforms and biosensing technologies that allow biomedical diagnostics from the gene level to single-cell, and whole body systems. Plasmonics refers to the research area of enhanced electromagnetic properties of metallic nanostructures that produce ultrasensitive and selective detection technologies. The technology involves interactions of laser radiation with metallic nanoparticles, inducing very strong enhancement of the electromagnetic field on the surface of the nanoparticles. These processes, often called 'plasmonic enhancements', produce the surface-enhanced Raman scattering (SERS) effect that could enhance the Raman signal of molecules on these nanoparticles more than a million fold. The SERS technology can be used to directly detect chemical species and biological species with exquisite sensitivity in environmental and biomedical samples.

A SERS-based nanoprobe technology, referred to as 'Molecular Sentinel' nanoprobes, has been developed to detect DNA targets of pathogenic agents (e.g., HIV) and biomarkers of diseases (e.g., BRCA1, ERB2 breast cancer genes). Other plasmonic platforms, such as gold nanostars, offer plasmon properties that efficiently transduce photon energy into heat for photothermal therapy. Nanostars, with their small core size and multiple long thin branches, exhibit intense two-photon luminescence, and high absorption cross sections that are tunable in the near infrared region with relatively low scattering effect, rendering them efficient efficient photothermal agents in cancer therapy. A theranostic nanoplatform construct was created, allowing SERS imaging and photodynamic therapy. SERS-based plasmonic nanoprobes and nanochip systems have also been developed for use as diagnostic systems for point-of-care personalized nanomedicine and global health applications.

In the field of biosensing of individual cells a unique advance has been the development of optical nanosensors, which have dimensions on the nanometer (nm) size scale. Using lasers as excitation sources for these nanosensors, it has become possible to probe physiological parameters (pH), biochemical species (DNA adducts) and monitor molecular pathways (apoptosis) in a single living cell. These nanosensors lead to a new generation of nanophotonic tools that can detect the earliest signs of disease at the single-cell level and have the potential to drastically change our fundamental understanding of the life process itself.

Spectrochemical detection using plasmonic nanomaterials and biosensing technologies are definitely bringing a bright future to biochemical and medical research and could ultimately lead to the development of new modalities of environmental sensing, early diagnostics, drug discovery, and medical treatment beyond the cellular level to that of individual organelles and even DNA, the building block of life.

9531-17, Session 3

Optical coherence elastography for depth-resolved biomechanical assessment of tissues

Kirill V. Larin, Univ. of Houston (United States)

The biomechanical properties of many tissues can have a profound influence on the health, structure, and normal function of the organism. Recently, optical coherence elastography (OCE) has been established for qualitative and quantitative noninvasive imaging of tissue elasticity. Reinforced from the high spatial resolution of optical coherence tomography (OCT), OCE is able to provide micro-scale depth-resolved mapping of tissue elasticity. Quantitative assessment of the elastic modulus of tissue has been demonstrated based on the use of OCE to monitor the propagation of the induced elastic waves in cornea, skin and soft-tissue tumor. More recently, our group has developed shear wave imaging OCT (SWI-OCT), which enables noncontact depth-resolved imaging and visualization of the elastic wave propagation inside tissue at an ultra-high frame rate. Obtained results indicate that the lamb wave dispersion curve agrees well with the analytical approximation and demonstrate the feasibility of using SWI-OCT to study the dispersion of lamb wave propagation in cornea. This allows depth-resolved quantification of tissue biomechanical properties without any contact.

9531-18, Session 3

Oil-based gel phantom for ultrasound and optical imaging

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Water-based materials are commonly used in phantoms for ultrasound and optical techniques. However, these materials have disadvantages such as easy degradation and low temporal stability. In this study we propose an oil-based new tissue mimicking material for ultrasound and optical imaging, with the advantage of presenting low temporal degradation. Styrene-Ethylene/Butylene-Styrene (SEBS) copolymer in mineral oil samples were made varying the SEBS concentration between 5?15%, and low-density polyethylene (LDPE) between 0?9%. Acoustic properties such as speed of sound and attenuation coefficient were obtained by the substitution technique with frequencies ranging from 2.25?10 MHz, and were consistent to that of tissue. These properties were controlled varying SEBS and LDPE concentration; speed of sound from 1445?1480 m/s, and attenuation from 0.55?1.0 dB/cm/MHz were observed. SEBS gels with 0% of LDPE were optically transparent, presenting low optical absorption and scattering in the visible region of the spectrum. In order to fully characterize the optical properties of the samples, the reflectance of the surface was measured, along with the absorption, scattering and albedo coefficients ranging from 400 nm to 1200 nm. The results suggest the copolymer gels are promising for ultrasound and optical imaging, what make them also potentially useful for photoacoustic imaging.

9531-19, Session 3

Quantitative mapping of retinal blood flow from dynamic fluorescein enhanced fluorescence imaging

Kenneth M. Tichauer, Micah Guthrie, Logan Hones, Lagnojita Sinha, Illinois Institute of Technology (United States); Keith St. Lawrence, Western Univ. (Canada); Jennifer J. Kang-Mieler, Illinois Institute of Technology (United States)

Accurate measures of retinal blood flow can serve as an indicator in many retinal diseases, such as diabetic retinopathy; however, quantitative methods of estimating blood flow have not been widely adapted to retinal blood flow imaging. In this study, a fluorescence imaging approach was employed to image dynamic changes in injected fluorescein contrast in the retina (and the arterioles feeding the retina). Various kinetic models were then applied to the image-based arterial input function and retinal fluorescein dynamics to extract quantitative estimates of blood flow (in volume of blood per time, per volume of tissue - ml/min/100g – a unit comparable from patient to patient. This work was carried out in healthy (n = 4) and streptozotocine-induced diabetic (n = 4; 4-5 weeks post-induction; mean blood glucose: 406 ± 121 mg/dL) rats. The mean retinal blood flow measures in healthy and diabetic rats, determined by the most robust blood flow measure (a plug flow model with no leakage), were 11.7 ± 1.2 ml/min/100g and 42.6 ± 25.9 ml/min/100g, respectively. The average increase in blood flow with diabetes, by a factor of 4, has been observed with relative blood flow approaches in prior studies, demonstrating the promise of this novel quantitative blood flow estimate. It should be noted that since fluorescein is FDA approved, and commonly used in ophthalmology, the optimal blood flow mapping methods realized by this work can be readily translated to the clinic to provide an early predictor of diabetic retinopathy, allowing earlier therapeutic intervention.

9531-20, Session 3

Retrieving the absorption coefficient of epidermis through the trigonometric parametrization of the diffuse reflectance curves

Freddy J. Narea, Aarón A. Muñoz Morales, Iraida Graterol, Univ. de Carabobo (Venezuela)

Human skin has been studied, given the interest in knowing information about physiology, morphology and chemical composition. These parameters can be determined using non invasive optical techniques in vivo, such as the diffuse reflectance spectroscopy. The skin phototype is determined by many factors, but primarily by the amount and distribution of the pigment melanin that is the principal chromophore absorption in the epidermis. The objective of this study is retrieve the absorption coefficient of the epidermis through the trigonometric parameterization of diffuse reflectance spectra . The sample consists of 50 healthy individual with different skin types according to the Fitzpatrick scale. The spectral data were fit with trigonometric series finding a direct dependence between of the independent term of the Fourier series expansion and absorption coefficient of the epidermis.

9531-21, Session 3

Clinical Study of ex vivo photoacoustic imaging in endoscopic mucosal resection tissues

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Accurate endoscopic detection and dysplasia in patients with Barrett's esophagus (BE) remains a major unmet clinical need. Current diagnosis use multiple biopsies under endoscopic image guidance, where up to 99% of the tissue remains unsampled, leading to significant risk of missing dysplasia.

We conducted an ex vivo clinical trial using photoacoustic imaging (PAI) in patients undergoing endoscopic mucosal resection (EMR) with known high-grade dysplasia for the purpose of characterizing the esophageal microvascular pattern, with the long-term goal of performing endoscopic PAI for dysplasia detection and therapeutic guidance. EMR tissues taken immediately from the patient were mounted on an agar layer and covered with ultrasound gel. Digital photography guided the placement of the PAI transducer, which operated at 40 MHz and scanned the luminal side of the specimen in 14 mm wide strips at 680, 750, 824, 850 and 970 nm. Acoustic images were simultaneously acquired. Tissues were then sliced and fixed in formalin for histopathology, including CD31/D2-40 staining. Ongoing analysis includes co-registration and correlation between the intrinsic PAI features and the histological images.

Preliminary PAI+ultrasound images have demonstrated the technical feasibility of this approach and point to the potential of PAI to reveal the microvascular pattern within the EMR specimens. There are several technical factors to be considered in rigorous interpretation of the PAI characteristics, including the loss of blood from the ex vivo specimens and the limited depth penetration of the PA signal.

9531-22, Session 3

Evaluation of the variable depth resolution of active dynamic thermography on human skin

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Active dynamic thermography (ADT) is an imaging technique capable of characterizing the non-homogenous thermal conductance of damaged tissues. The purpose of this study was to determine optimal stimulation parameters and quantify the optical resolution of ADT through various depths of human skin. Excess human skin from plastic surgery operations was collected immediately following excision. A total of 12 thin split-thickness (0.010"), thick split-thickness (0.024") or full thickness (0.030") grafts were harvested from four patients. Grafts were placed on top of a custom 3D printed resolution chart with line spacing ranging from 0.445-0.125 line-pairs per millimeter. Thermographic stimulation was applied from a 300W halogen lamp array for between 0.5-10 seconds to determine optimal parameters at each depth of skin. Video was captured with a thermal camera, and analysis was performed with custom software. In this study ADT resolved 0.387±0.044lp/mm at a depth of 0.010", 0.326±0.035lp/mm at a depth of 0.024" and 0.238±0.056lp/mm at a depth of 0.030". The stimulus energy required for maximum resolution at each depth was 4s, 8s and 10s respectively. ADT is a sensitive technique for imaging dermal structure, capable of resolving detail as fine as 1,322 ?m, 1,593 ?m and 1,982 ?m in thin and thick partial and full-thickness grafts respectively. This study has characterized a correlation between stimulus input and maximal resolution at differing depths of skin. It has also defined the functional imaging depth of ADT to below the sub-cutis, well below conventional spectrophotometric techniques.

9531-24, Session 4

A simple dental caries detection system using full spectrum of laser-induced fluorescence

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Conventional methods for detection of caries present high specificity but low sensitivity and reproducibility, mainly due subjectivity. The aim of this work was develop an apparatus for the detection of early caries lesions in enamel using the full extent of the tooth fluorescence spectrum, through the integration of a laser diode, fiber optics, filters and one portable spectrometer connected to a computer, all commercially available; to evaluate the developed device in clinical and laboratory tests, and compare its performance with commercial equipment. Methods: clinical examinations were performed in patients with indication for exodontics of premolars. After examinations, the patients underwent surgery and the teeth were stored individually. The optical measurements were repeated approximately two months after extraction, on the same sites previously examined, then histological analysis was carried out. Results: the spectral detector has presented high specificity and moderate sensitivity when applied to to differentiate between healthy and damaged tissues, with no significant differences from the performance of the commercial equipment. The developed device is able to detect initial damages in enamel, with depth of approximately 300 ?m. Conclusions: we successfully demonstrated the development of a simple and portable system based in laser-induced fluorescence for caries detection,

assembled from common commercial parts. As the spectral detector acquires a complete recording of the spectrum from each tissue, it is possible to use it for monitoring developments of caries lesions.

9531-25, Session 4

Cadmium-free quantum dot nanoparticles as a novel fluorescence probe for in vivo imaging

Elnaz Yaghini, Univ. College London (United Kingdom); Helen Turner, Imad Naasani, Lesley Smith, Nanoco Technologies Ltd. (United Kingdom); Alexander J. MacRobert, Univ. College London (United Kingdom)

Quantum dot (QD) nanoparticles are being explored as fluorescent probes for biomedical imaging. Our aim was to develop new biocompatible cadmium-free QDs and study their potential as a novel fluorescence probe under in vivo condition and also investigate their in vivo toxicity

For the pharmacokinetic study, water soluble cadmium-free QDs were administered intravenously into the healthy Hooded Lister rats. Animals were sacrificed at various times postinjection and major organs from each rat were harvested. The QD biodistribution was investigated by elemental analysis using inductively coupled plasma mass spectroscopy (ICP-MS). The uptake of QDs in tissues was also examined by ex vivo CCD fluorescence imaging. Standard haematological and biochemical markers were used to evaluate QD-induced toxicity. Histological assessment of tissues was also performed.

Using ICP-MS, the results showed that QDs predominantly accumulated in the liver and spleen as would be expected for a nanoparticle of this size. The overall level of QDs in other tissues remained rather low. The QD level in tissues decreased over time, suggesting their excretion from the body. Additional evidence for the uptake of QDs in tissues was obtained by ex vivo fluorescence imaging. The haematological and biochemical markers remained within the normal ranges after 30 days and histology of the major organs showed no abnormalities. Overall, we report the development of biocompatible cadmium-free QDs with an excellent quantum yield (QY) for in vivo imaging without significant in vivo toxicity. This study supports the potential role for QDs in clinical applications, such as image-guided surgery for tumour removal.

9531-26, Session 4

Optical diagnostic and photodynamic therapy of early stage cancer using double integrating sphere

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The treatment of cancers with photodynamic therapy (PDT) relies on uniformly covering the treatment target with sufficient light fluence using an externally applied source. Inhomogeneities in the irradiance of light from the patient's tumer can introduce dose uncertainties that prevent an optimum treatment. These can be reduced by incorporating an integrating sphere into the treatment delivery.

In our experiment, cancerous and non-cancerous blood serum was exposed at irradiance of 0.1 W/cm2 by using He-Ne laser in conjunction with double integrating sphere system. The results of forward and backscattering intensity of normal and malignant serum show an exponential decrease in the fluorescence amplitude. The experimental results indicate that there is notable amplitude difference between the malignant and normal blood serum showing decreased scattering in malignant blood serum. These results have important implications in photo-diagnosis and photodynamic therapy.

9531-27, Session 4

Combined phosphorescence-holographic approach for singlet oxygen detection in biological media

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Investigations on photodynamic generation of singlet oxygen are very important for a wide variety of photochemical and photobiological processes. In vacuum the radiation transition from the singlet state to the ground triplet state of the oxygen molecule at 1270 nm is strongly forbidden, the quantum efficiency of the phosphorescence signal is very low and most of the excited energy is released through radiationless processes causing local excitation of surrounding molecules and formation of a thermal lens. Holographic interferometry allows one to obtain in a single shot a 2D image of the area under study containing information on spatial distribution of local variations of refractive index induced by temperature variations. The opportunity to obtain data in time domain is provided by the technique of digital holography.

In the present paper we suggest a combined approach providing simultaneous recording of radiative and radiationless deactivation of singlet oxygen molecules by means of direct registration of phosphorence at the wavelength of 1270 nm and recording of spatial distributions and temporal evolution of thermal disturbances by means of digital holography. The data obtained provides a complementary and complete set of information on singlet oxygen location and behavior in the medium. Experiments demonstrating feasibility of the approach are performed on the monitoring of photosensitized generation and deactivation of singlet oxygen in water.

9531-28, Session Pln3

Acoustic radiation force optical coherence elastography *(Invited Paper)*

Zhongping Chen, Beckman Laser Institute and Medical Clinic (United States)

We report on the development of a resonant acoustic radiation force optical coherence elastography (ARF-OCE) technique that uses mechanical resonant frequency to characterize and identify tissues of different types. Knowledge of tissue mechanical properties provides valuable medical information in disease diagnosis and prognosis. There is a close correlation between tissue elasticity and pathology. For example, in atherosclerosis, measurement of tissue biomechanical properties has the potential to differentiate between various plaque components. Furthermore, tissue mechanical properties provide critical information to assess the vulnerability of plaques. The stress in the cap increases with decreasing thickness and increasing macrophage infiltration. High strain locations in the vessel wall indicate the presence of vulnerable plaques. We have applied the ARF-OCE to image post-mortem human coronary artery with atherosclerosis. The result demonstrates the potential of ARF-OCE as a non-invasive method for imaging and characterizing vulnerable plaques. The ARF-OCE will have a broad range of clinical applications, including imaging and characterizing cardiovascular atherosclerotic lesions, imaging and diagnosing of early stage cancer, and imaging and evaluating age-related macular degeneration (AMD).

9531-29, Session 5

Applications of long-range optical coherence tomography in the head, neck, and upper airway *(Invited Paper)*

Brian J Wong M.D., Giriraj Sharma M.D., Bryan Lemieux, Joe C Jing, Max Wiedmann, Zhongping Chen, Beckman Laser Institute and Medical Clinic (United States)

This presentation will review the use of OCT in head and neck imaging at our institution. We will discuss briefly our transition first from time domain to spectral domain OCT systems, and then progression of using long-range systems including those using VCSEL sources. Applications will focus on the use of this technology platform in examining the upper airway with an emphasis sleep disordered breathing and subglottic stenosis. In addition, we will discuss the use of specialized probes and scanning systems required to image these anatomic sites.

9531-30, Session 5

Blood vessels detection during brain needle biopsy using a Monte Carlo based sub-diffuse tomography algorithm

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One of the main risks associated with brain needle biopsies is hemorrhaging due to clipping blood vessels during tissue extraction. This procedure is necessary in order to determine the pathological nature of lesions (e.g. brain tumors) in order to inform the choice of treatment, possibly including surgical resection. In order to improve safety, we present a new optical spectroscopy technique using multiple diffuse reflectance signals to detect blood vessels based a 360-degrees reconstruction of the tissue surrounding the needle in terms of the optical contrast associated with hemoglobin absorption.

Monte Carlo Simulations (MCS) are performed to evaluate the sensitivity of the algorithm and to optimize the imaging geometry. Full tomographic data are obtained by simulating the different laser injection points with their respective detection points, with appropriate pre-defined source and detector sizes and numerical apertures. The optical needle geometry is modeled by a tetrahedral mesh and simulations are performed using Mesh-based Monte Carlo (MMC). Different cases (12, 24, 32 fibers) are studied. Their performance is evaluated with MMC for realistic blood vessel distributions using 3D computed tomography images of pig brains. Blood vessel detection is achieved via a novel approach exploiting rescalable MCS to recover the absorption based on computed photon path lengths. A least mean square approach for non-linear systems is used to minimize the discrepancy between measured and computed sinograms. The method can recover the absorption heterogeneities as well as the concentration of oxygenated and deoxygenated haemoglobin using multispectral data.

9531-31, Session 5

Radiation dosimetry imaging through gated optical detection of Cerenkov emission

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Cerenkov light emission from gamma-ray and electron beam delivery to water tanks has recently been shown to be detectable and could be used in some applications for 3D dosimetry in radiation therapy. The major potential benefit of Cerenkov imaging is that it is a way to image beams in real time with over 30 frames per second. As such, it is feasible to image IMRT and VMAT treatment beams in water tanks dynamically, and create composite visualizations of the treatment plans. This imaging can be used to verify new treatment plans prior to application to patients, or to quickly verify new machines, or testing in situations where access is limited. The strength of this optical imaging is that it is simple to implement, and provides immediate feedback. The drawbacks are related to minor differences between Cerenkov emission and dose, which make it not an exact measurement of dose.

The situations where there is a high degree of linearity between Cerenkov emission and Dose deposited are when 1) imaging broad surfaces, imaging lower energy beams, imaging symmetric treatment plans. Even in the cases where there is not good linearity, there is potential to calibrate the ratio based upon well-known estimates from Monte Carlo simulations or from measurements.

In human imaging studies, two clinical trials have been completed to image surface emissions in real time during therapy. In the first case, whole breast irradiation was followed for fractionated therapy in 12 patients. Positioning repeatability was within the 3mm distance to agreement criteria, and the Cerenkov images were able to be used to verify patient alignment. In the second trial, total skin irradiation was followed in 2 subjects and it was found that the irradiation pattern commonly used was not as homogenous as would be desired, with a 25% variation found from the midline to the feet. This was corrected based upon whole body Cerenkov imaging.

Future potential uses for Cerenkov will be discussed, based upon the strengths and weaknesses of the imaging system as a tool for medical physics or clinical radiotherapy.

9531-32, Session 5

Evaluation of eye tissue elasticity by means of sound propagation velocity measuring in vivo

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Introduction: To date, it has never been demonstrated the propagation speed of sound in human corneas and lens in vivo. With the advent of Optical Coherence Tomography (OCT), it became possible to determine the dimensions of the ocular tissues without the interference of sound propagation speed and to use this information to define the real propagation speed of sound for each patient and individualized structure. Aim: To determine the sound propagation speed in the cornea and lens from patients that theoretically exhibits differences in tissue elasticity (normal corneas and keratoconus, corneas of young and elderly patients, in addition to lens from young and elderly patients). Then, relate the determined velocity in each group with the expected tissue elasticity of the cornea and lens. Methods: We studied 100 eyes from 50 patients: 50 with keratoconus and no cataract and 50 with cataract and no corneal changes. All patients measured corneal and lens thickness by ultrasound methods (Ultrasonic Biomicroscopy - UBM and Ultrasonic Pachymetry - UP) and by OCT (RTVue®, Lenstar® and Visante®), then were divided

into 2 groups: Group 1 (Cornea) analyzed the central corneal thickness (UBM, UP, RTVue®, Visante®, Lenstar®); Group 2 (Lens) analyzed the axial thickness of the lens (UBM and Lenstar®). Based on standard ultrasonic velocities from UP (1640m/s) and UBM (1548m/s), we calculated the real propagations speed of sound in each tissue. Results: Based on UP, the corneal sound speed on control group (1616m/s) was faster than on keratoconus group (1547m/s) (P<0.0001). Based on UBM, the lens sound speed on cataract group (1664m/s) was faster than on control group (1605m/s) (P<0.0001). Discussion: It is known that sound propagates faster in materials with lower elasticity. We found that the sound speed on keratoconic corneas (high elasticity) was slower and on cataract lens (lower elasticity) was faster than normal corneas and lens, respectively, in vivo.

9531-33, Session 5

Fluorescence spectroscopy for assessment of liver transplantation grafts concerning graft viability and patient survival

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Evaluating transplantation grafts at harvest, when organ viability must be assured, is essential. Laser-induced fluorescence spectroscopy (LIFS) is a non-invasive technique that can help monitoring changes in metabolic/structural conditions of tissue during transplantation. We aim to correlate LIFS-obtained spectra of human hepatic grafts during liver transplantation with both patients' mortality in early post-operative and biochemical parameters, establishing a method to exclude non-viable grafts before implantation. Fifteen patients underwent orthotopic liver transplantation, piggyback technique (9 survived after transplantation). LIFS used 408nm excitation laser, spectrophotometer (350-850nm), and optical fiber to generate and collect fluorescence. Liver spectra were obtained for lateral left, lateral right, and medial right lobes. Collection was performed immediately after opening donor's cavity (time T1), after cold perfusion (T2), end of back-table (T3), and 5min and 1h after warm perfusion (T4 and T5, respectively). Spectra were analyzed by comparing ratios between amplitudes (442,469,507,556,605,685nm) among themselves, and with lactate, creatinine, bilirubin and INR levels. LIFS was sensitive to liver changes during transplantation. For 78% of survivors, ratios differ for each stage but follow a regular trend, which does not happen for 67% of deceased patients. The relative variations in lactate plus INR present small variation (below 2.5) for all survivors, while the same 67% of deceased patients showing fluorescence irregular trends present large variation (above 2.5), revealing a relation between LIFS and biochemistry. This study is in progress; initial results indicate correlation between fluorescence and life/death status of patients.

9531-34, Session 5

Trans-rectal diffuse optical tomography to monitor photocoagulation during interstitial photothermal therapy of focal prostate cancer

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Near-infrared interstitial photothermal therapy is currently undergoing clinical trials as an alternative to watchful waiting or radical treatments in patients with low/intermediate-risk focal prostate cancer. Transrectal diffuse optical tomography (TRDOT) is being developed to directly image the photocoagulation boundary based on tissue optical property changes, and hence to determine the completeness of target tumor destruction while avoiding damage to the rectal wall. Forward simulations were performed using NirFast to determine the sensitivity of changes in the optical signal resulting from a growing coagulated lesion with optical scattering contrast, for varying light source-detector separations in both longitudinal and transverse imaging geometries. The simulations were validated experimentally in tissue-simulating phantoms using an existing continuous-wave TRDOT system, in a configuration that is representative of one potential intended clinical use. In parallel, a shape-based DOT algorithm is in development, with optical properties of the prostate and coagulation lesion assumed to be different but constant, while parameters defining the lesion shape (assumed to be ellipsoidal) varied and optimized to match the measured optical signal. Simulations demonstrate the feasibility of this approach and it's robustness to differences between actual and assumed optical properties. These measurements and simulations provided critical guidance for the optimum design of the transrectal applicator probe, in achieving maximum sensitivity to the presence of the coagulation boundary and, consequently, the highest accuracy in determining the boundary location relative to the rectal wall. Two TRDOT probes are now being assembled to be used simultaneously with either MRI or ultrasound for anatomical imaging.

9531-35, Session 6

Conjugated polymer sensors for biology and medicine *(Invited Paper)*

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Conjugated polymers are an important class of organic semiconductor that combine novel optoelectronic properties with simple processing from solution. They are widely researched for applications in displays, solar cells and flexible electronics, and we have also demonstrated a wearable light source for photodynamic therapy of skin cancer. Here we demonstrate their potential in biophotonics by making wearable sensors for tissue oxygenation and muscle contraction. The tissue oxygenation sensor consists of an organic light-emitting diode (OLED) and two photodiodes, and its operation has been demonstrated by the measurement of forearm ischaemia. The muscle contraction sensor consists of an OLED and four photodiodes, and can distinguish between isotonic and isometric muscle contractions. We show that it can be used to control a robot arm, demonstrating its potential to control prosthetic limbs. Our results show that organic semiconductors have great potential for wearable sensors in biology and medicine.

9531-36, Session 6

Android phone controlled compact imaging system for biosensing applications

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We present a compact imaging device that interfaces with an android phone for detection of cells and biomolecules. This device features integrated optics and electronics for imaging using LED white light and red diode laser. Optics is laid out in a similar way as in the optical pickup of compact disk drives, however the components are packaged in a custom designed and 3D printed casing. The device is powered by a rechargeable battery and the control unit consists of a microcontroller board which provides all necessary communication as well as control functions. A high resolution CMOS image sensor interfaced with the controller board acquires images and transfers to the phone for processing. An application program running on the phone performs data acquisition, processing and control of the device like any other touch-based android application.

Cell and biomolecule samples, prepared on bar-coded strips, are inserted into the device to activate it and initiate communication with the phone. A live image of the strip is shown when the user opens the application. The bar-code is also read to load relevant recipe and the system also populates the user interface with appropriate icons. Upon analysis, the user can readily share the results through email or other data sharing

functions integrated into the application. The main goal of this system is to completely isolate the user from the underlying complexity of regular imaging and data analysis systems and/or experimental setups that usually require trained personnel.

We demonstrate application of the imaging system for cells, beads and gratings. Our findings suggest that this device can be used for medley of optical biosensing techniques.

9531-37, Session 6

Compact handheld multispectral fluorescence lifetime imaging (FLIM) endoscope for in vivo imaging of oral cancer

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A compact handheld system for simultaneous multispectral FLIM imaging based on a MEMS scanner and small diameter (6.25 mm) lenses is presented. The handheld endoscope consists of a compact enclosure (10 X 5 X 3 cm3 in volume) with a rigid probe (0.8 cm diameter, 12 cm length). The customized enclosure holds the MEMS scanner and allows slightly angle adjustment for a dichroic mirror placed at 45° angle to the incoming excitation beam. The rigid probe includes four achromatic lenses (f = 30mm). The first two lenses form a relay system to extend the length of the probe. The most distal lens works as an objective to focus the light onto the sample. An additional lens is placed in the intermediate image plane of the relay system to serve as a field lens which increases the FOV from ~3.6 mm to ~5 mm. The system lateral resolution is ~100 ?m measured by imaging an USAF target. The time-resolved fluorescence emission is spectrally divided in three emission bands and multiplexed in time following a strategy previously reported. Thus, for a single excitation pulse, multiple decays corresponding to different spectral bands can be recorded using a single detector. The multispectral fluorescence signal is detected by an MCP-PMT and digitized at 6.25 GS/s. Real-time image processing is accomplished by embedding FLIM deconvolution within the imaging instrumentation. A pixel acquisition and processing of 10 kHz is demonstrated. The system is validated by imaging standard fluorescent dyes, oral biopsy ex vivo and human oral mucosa in vivo.

9531-38, Session 6

Exploring automatic optical tweezers system on the evaluation of erythrocytes elasticity

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Optical tweezers have been used to trap, manipulate and measure individual cells properties. In this work we show that the association of computer controlled optical tweezers system and image processing techniques allows rapid and reproducible evaluation of cell deformability. In particular, the deformability of red blood cells (RBCs) plays a key role in the transport of oxygen through the blood microcirculation. The automatic measurement processes consisted of three steps: acquisition, segmentation of images and measurement of the elasticity of the cells. An optical tweezers system was set up on an upright microscope equipped with a CCD camera and a motorized XYZ stage, computer controlled by a Labview platform. On the optical tweezers setup, deformation of the capture RBC was obtained by moving the motorized stage. The automatic real-time homemade system was evaluated by measuring red blood cells elasticity from normal donors and patients with thalassemia and sickle cell anemia. Approximately 150 erythrocytes were examined, and the elasticity values obtained using the developed system were compared to the values measured by two experts. With the automatic system there was a significant time reduction (100x) on the erythrocytes elasticity evaluation. Automated system can help to expand the applications of optical tweezers in hematology and hemotheraphy.

9531-39, Session 6

A compact multi-wavelength optoacoustic system based on high-power diode lasers for biomedical applications

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Many clinical applications of optoacoustic systems require a multi-wavelength nanosecond pulsed laser source that is compact and light-weight, while providing sufficient pulse energy and repetition rate. The use of diode lasers combines many specific advantages, which make them very attractive for optoacoustics in biomedical applications. Diode lasers are small enough, so that many of them could be combined in a system, without increasing its size as their solid-state laser source competitors do. This permits a high flexibility to build up special geometries to realize the best suitable light source for any particular optoacoustic application. The high repetition rates of diode lasers are also a strong advantage. While Nd:YAG-based systems are working at 10 Hz frequency repetition rate, diode lasers can be driven at up to a few kHz with high energy pulses.

In our experiments, an array of diode lasers fed by custom-built driving circuits and operating at different wavelengths is employed as excitation source to generate broadband ultrasonic waves. Each diode laser is driven by a driver designed to provide variable pulse duration and repetition rate. The multi-wavelength excitation is combined in an optical fibre bundle and is projected on the target. The optoacoustic signal produced by the transient pressure occurring in the target is detected by a piezoelectric transducer and is externally processed by using proper algorithms for image reconstruction. In this work, we report the use of this system to optimize the detection sensitivity of nanoparticle solutions immersed in a phantom gel, mimicking the optical behavior of a turbid biological tissue used as a target to evaluate the generated OA signals.

9531-41, Session 6

Development of an in situ controllable polymerization tool and process for hydrogel used to replace nucleus pulposus

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Currently implants or tissue replacements are inserted either as a whole implant or by injecting a liquid which polymerizes to form a solid implant at the appropriate location. This is either highly invasive or not controllable. We developed a tool to perform such surgeries in a minimally invasive and controllable way. It combines photopolymerization and fluorescence spectroscopy in a surgical apparatus. However, to successfully replace tissue such as cartilage or an intervertebral disc, photopolymerizable materials do not only need to be photoactive. They should also be able to withstand the environmental loading conditions

after implantation.

Therefore we developed a set of in situ and in vitro tests adapted to the evaluation of photopolymerized tissue replacements and implants. Highly tunable Poly-Ethylene-Glycol (PEG) hydrogels were successfully implanted into a bovine caudal model and mechanically tested in vitro during two weeks. The hydrogels were evaluated at the tissue level (adherence and mechanical properties of the implant), at the cellular level (biocompatibility and cytotoxicity), and at the ergonomic level (sterilization procedure and feasibility study).

In vitro the device and hydrogel were successfully tested to replace the core of intervertebral discs. It was possible to conduct surgeries through a hole smaller than 1 mm in diameter while current state of the photopolymerization could be monitored in real time. Furthermore, the tested PEG hydrogel showed similar material properties in terms of swelling and adherence compared to native intervertebral disc tissue. It could be shown that the hydrogel, the liquid precursor, and the illumination light to induce the photopolymerization are biocompatible with human intervertebral disc cells.

9531-23, Session PSun

Innovative parameters obtained for digital analysis of microscopic images to evaluate in vitro hemorheological action of anesthetics

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Drugs used during anesthesia could enhance microvascular flow disturbance, not only for their systemic cardiovascular actions but also by a direct effect on the microcirculation and in particular on hemorheology. This is particularly important in high-risk surgical patients such as those with vascular disease (diabetes, hypertension, etc.) or who presented high predisposition to develop shock and oxygen deficiency. When blood is at rest, erythrocyte aggregates are forming "stacks of coins" or rouleaux. In pathological conditions, such as an increased content of plasma proteins, alterations of red cell surface charge or a decrease in speed of movement, aggregate formation, change in the morphology of the aggregates and resistance to disaggregation can be promoted. Since the erythrocyte aggregation characteristics vary between healthy donors and patients with vascular or hematological diseases (diabetes, hypertension, etc.) and that patho-hemorheologic and pato–hemostasiologic changes can be detected before and increased during surgery, it is important to take preventive actions before surgery to obtain the maximum benefit for the patient. It has been found that propofol has side cardiovascular effects such as decrease in peripheral vascular resistance, decreased venous oxygen saturation jugular, induced hemolysis, and even more severe, it can cause the so-called propofol infusion syndrome, a rare but lethal condition. Recent studies found that propofol interacts with the erythrocyte membrane without affecting the blood or plasma viscosity also decrease platelet aggregation, but at concentrations above those achieved during surgery (steady-state). Therefore, in this work we propose a set of innovative parameters obtained by digital analysis of microscopic images to study the in vitro hemorheological effect of propofol and remifentanil on red blood cell from type 2 diabetic patients compared to healthy donors. Obtained innovative parameters allow quantifying erythrocyte aggregation alterations, which can increase the in vivo risk of microcapillar obstruction.

9531-116, Session PSun

In-vivo, percutaneous, needle based, optical coherence tomography of renal masses

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Introduction: Optical coherence tomography (OCT) is an imaging technique, based on the backscattering of near infrared light. Within OCT images the attenuation coefficient (μOCT) can be calculated, expressing the loss of signal per millimeter of tissue penetration. During carcinogenesis cellular changes take place that alter tissue scattering properties. We hypothesize that the μOCT is higher in malignant versus benign tumours, providing the means for tumour differentiation. The aim of this study is developing OCT into an instant and accurate technique for tumour differentiation.

Materials and methods: Percutaneous, needle based, OCT of the kidney was performed in patients presenting with a solid renal mass. In the same session, conventional core biopsies were harvested. Additional pathology specimens, resection or biopsy, were acquired during final treatment. Subsequently μOCT values were correlated to histopathology results.

Results: 49 renal tumours were included, resulting in 46 OCT scans. The first 10 cases show a difference in the μOCT between benign tissue (AVG 2.86 mm-1, STDV 0.99) and malignant renal masses (AVG 3.90 mm-1, STDV 1.70). Qualitative analysis of OCT scans proved valuable in confirming successful targeting. The remaining cases will analysed upon availability of final pathology results, which is expected in the near future.

Conlusion: Percutaneous, needle based, OCT of renal masses is safe and easy. Analysis of the first 10 cases show a distinct difference in μOCT between benign tissue and malignant renal masses, providing the means for tumour differentiation.

9531-117, Session PSun

Fluorescence diagnosis of upper respiratory tract infections

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The pharyngitis and laryngitis are respiratory tract infections highly common. Pharyngitis can be accompanied by fever, especially if caused by a systemic infection. Laryngitis is an inflammation of your voice box (larynx) from irritation or infection. The conventional treatment is the antibiotics administration, which may be responsible by an increase of identification of bacterial strains resistant to drug. This fact associated to high incidence these infections become important to develop new technologies for diagnosis. This study aims to evaluate the use of fluorescence spectroscopy for the characterization of oropharynx infections, in order to diagnose the bacteria colonization. The imaging system for wide field fluorescence visualization is Evince (MMOptics, São Carlos, SP, Brazil) coupled to a Apple iPhone®. The system consists of Light Emitting Diodes (LEDs) operating in the violet blue region centered at green-red spectrum 450 nm and optical filters that allow viewing of fluorescence. A tongue depressor was adapted to Evince for mouth opening. The same images were captured with white light and fluorescence with a optical system. The red fluorescence may be an bacterial marker for physiological monitoring of oropharynx infection processes. The bacterial biofilm on tissue were assigned to the presence of protoporphyrin IX. This work indicates that the autofluorescence of the tissue may be used as a non-invasive technique to aid in the oropharynx infection diagnostic.

9531-118, Session PSun

Plasmonic enhancement in the photoinactivation of Escherichia Coli using Rose Bengal and gold nanoparticles

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In the present study we report on the ability of gold nanoparticles (AuNP) to enhance the antimicrobial activity of the photosensitizer Rose Bengal (RB), a very effective singlet oxygen generator. Our experiments were conducted using a suspension of Escherichia Coli in the presence of either RB or a combination of RB and AuNP. Nanoparticles were synthesized by laser ablation in water, which allows high purity, biologically friendly AuNP production, as compared to traditional chemical methods. Several relative concentrations of bacteria, photosensitizes and AuNP were studied. Bacterial survival rates were determined before and after LED light illumination. The phototoxicity of RB with and without AuNP was checked following illumination for 10 and 20 minutes. As a control, the dark toxicity of RB was verified. The results show that the survival rate of bacteria decreases significantly with the increase of RB concentration and illumination time, which is in accordance with previous works. Interestingly, our results also indicate a significant increase in the lethal photosensitization of RB in the presence of AuNP. We propose this effect is due to plasmonic light enhancement, considering the superposition of RB and AuNP absorption spectra, which favors electric field enhancement effects in the presence of AuNP. Similar experiments using the photosensitizer Methylene Blue (MB) allowed us to test our hypothesis for MB did not show any difference in its phototoxicity in the presence of AuNP. We propose this observed synergistic effect could be an effective way for improving photodynamic inactivation of microorganisms.

9531-119, Session PSun

Fluorescent liposomes to probe how DOTAP lipid concentrations can change red blood cells homeostasis

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Liposomes have been used to deliver DNA, and more recently, nanoparticles such as quantum dots, into living cells. Their electrostatic interaction with the cell's surface (negatively charged) can lead to membrane destabilization and/or fusion, facilitating intracellular release of those compounds. Nevertheless, cationic lipids can modify living cells homeostasis, depending on their concentration. In this study, we observed that the DOTAP cationic lipid concentrations influence in the red blood cells (RBCs) homeostasis by using fluorescent fusogenic liposomes consisted of three lipids: DOPE, DOTAP and DMPE-Rhodamine (1:1:0.1 mM respectively), varying DOTAP ratios from 0.1 to 1 mM. We used RBCs (1% in saline) to probe liposomes ability to fuse with cells, since they possess a surface very sensitive to charge variations and do not perform endocytosis. Liposomes were characterized by zeta potential and dynamic light scattering (DLS) and their interaction with RBCs was evaluated by fluorescence microscopy and flow cytometry. Zeta potential results showed that, from 0.3 to 1 mM concentration, the charge does not change significantly. The liposomes' diameter increases when more DOTAP was added, according to DLS results. Flow cytometry and microscopy analysis showed that at the DOTAP's highest concentration the liposomes fusion was faster than at the lowest concentration, however it was also more harmful to cells. Thus, the results showed that it is possible to use lower concentrations of DOTAP keeping the fusogenic liposomes's ability and cell homeostasis. This is important to guarantee a greater efficiency for delivery nanoparticles or other active samples into cells.

9531-120, Session PSun

Analysis of photodynamic cream effect in dental caries using optical coherence tomography

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Objective: The aim of this study was to assess the effect in the enamel demineralization of low-intensity infrared laser (?=810?nm, 100 mW/cm2, 90?sec, 4.47?J/cm2, 9?J) with or without photodynamic cream fluorinated or not fluorinated, using Optical Coherence Tomography (OCT). Background data: Lasers can be used as tools for the prevention of tooth enamel demineralization. Methods: All enamel specimens (n= 105) were analyzed using OCT at baseline, and randomly assigned into seven groups (n=15): C (+), laser application; C(-), no treatment; (F), acid fluoride gel; cream (IV); cream and neutral fluoride (IVF); cream and laser (IVL); and cream with neutral fluoride+ laser (IVFL). The specimens were submitted to all kind of treatments before demineralizing pH cycling challenge and were reanalyzed. Results: ANOVA and Tukey's multiple comparative analysis ($p < 0.01$) demonstrated a greater delta attenuation between baseline and post challenge for C + (0.034 ± 0.011) compared to IVF (0.016 ± 0.007) F (0.018 ± 0.010) IVFL (0.019 ± 0.008), and IVL (0.014 ± 0.010). The cream laser group (IVL) also showed lower delta (0.014 ± 0.010) compared to C - (0.025 ± 0.008). Conclusions: The OCT technique demonstrated that cream associated with laser showed the lowest quantitative enamel mineral looses after cariogenic challenge.

9531-121, Session PSun

Micro energy-dispersive x-ray fluorescence spectrometry study of dentin coating with nanobiomaterials

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New biomaterials such as multi-walled carbon nanotubes oxide/graphene oxide (MWCNTO/GO), nanohydroxyapatite (nHAp) and combination of them together or not to acidulated phosphate fluoride gel (F) were tested. Fourteen bovine teeth were selected and had two sites of pre-treatments: acid etched area and a control area. After the biomaterials application, the samples were submitted to six pH cycles. Micro energy-dispersive X-ray fluorescence spectrometry (μ-EDXRF) mapping area analyses were performed on both sites (n=84). Artificial saliva and MWCNTO/GO/nHAp/F composite treatments produced lower dentin demineralization than in the other groups. MWCNTO/GO/nHAp/F composite treatment resulted in levels of demineralization similar to the control group.

9531-122, Session PSun

The decontamination of oral mouth by antimicrobial photodynamic therapy

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Perform decontamination of the oral cavity prior to different dental procedures is fundamental in order to minimize the risk of cross infection in dental offices. Different agents and techniques can be used for this purpose, and then chlorhexidine has often used as antimicrobial agent.

However, chlorhexidine has some disadvantages and then it is necessary to test other antimicrobial agents or techniques. The antimicrobial photodynamic therapy (aPDT) promotes bacterial death as a result of photosensitization of microbial components, and it is a technique that involves the activation of photosensitizers by light source with a specific wavelength in the presence of molecular oxygen, resulting in the production of different radicals capable to induce the death of microrganisms. Thus, aPDT could be used in order to promote the decontamination of the oral cavity prior to surgical or non-surgical procedures in dental offices. Then, the purpose of this study was to evaluate the susceptibility of pathogens found in human saliva after sensitization by aPDT using curcumin exposed to blue light at 450nm. A randomized clinical trial was conducted with 10 adult volunteer patients (Approved by Human Ethic Committee, Araraquara School of Dentistry-UNESP). Three Groups were investigated: Group L0D0 (no light ,no photosensitizer, Control Group), chlorhexidine Group (treated with chlorhexidine) and Group L+ D+ (treated with aPDT using curcumin at 20?M activated by blue light. Samples of unstimulated saliva were collected prior and after the application of different techniques. Serial dilutions were performed and the resulting samples were cultured on blood agar plates at microaerophilic atmosphere at 37°C for 48 hours. The colony forming units (CFU/mL) were then determined. To analyze the data distribution the Kolmogorov-Smirnov test was used. As the data showed a normal distribution, the paired t Student's test was used to verify whether there were differences between the groups evaluated . Statistically significant difference was observed between Chlorhexidine Group and Group L + D + after applying the same ($p<0.05$). When the colony forming units (CFU / mL) compared to Group L + D + (before and after), we observed a significant decrease, with the order of a log ($p <0.05$). For the Chlorhexidine Group (before and after), no significant statistical differences ($p> 0.05$). Thus, the analysis of the results of this work, the reading of Colony Forming Units, allows us to conclude that photodynamic therapy had better bactericidal action toward solving chlorhexidine digluconate.

9531-123, Session PSun

Assembly and characterization of a fluorescence lifetime spectroscopy system for skin lesions diagnostic

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The fluorescence spectra and fluorescence lifetime analysis in biological tissues has been presented as a technique of great potential for tissue characterization for diagnostic purposes. The objective of this study is to assemble and characterize a fluorescence lifetime spectroscopy system for the diagnostic of clinically similar skin lesions in vivo. The fluorescence lifetime measurements were performed using the Time Correlated Single Photon Counting (Becker and Hickl, Berlin, Germany) technique. Two lasers, one emitting at 378 nm and another at 445 nm, are used for excitation with 20, 50 or 80 MHz repetition rate. A bifurcated optical fiber probe conducts the excitation light to the sample, the collected light passes through bandpass filters and goes to a hybrid photomultiplier tube detector. The fluorescence spectra were obtained by using a portable spectrometer (Ocean Optics USB-2000-FLG) with the same excitation sources, with an addition of a 405 nm excitation laser. An instrument response function of about 300 ps was obtained and the spectrum and the fluorescence lifetime of a standard fluorescent molecule (rhodamine) was measured for the calibration of the system ((4.1 ± 0.3) ns). The assembled system was considered robust, well calibrated and will be used for measurements of clinical skin lesions.

9531-124, Session PSun

In vivo quantitative tumor classification using targeted SERS nanoparticles

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Three out of four cases of lung cancer are diagnosed at a late stage for which the survival rate is very poor, so improving early detection is critical. Since lung cancer is a very heterogeneous disease, a spectrum of molecular markers is needed to adequately classify a tumor to determine optimal management. The development of surface enhanced Raman scattering (SERS) nanoparticles (NPs) that have narrow (1-2nm) spectral peaks and have bright enough Raman signals for endoscopic detection offers the possibility of high-level multiplexed biomarker imaging. Here we show that functionalized SERS NPs are able to bind to cell surface markers using a brief topical application in vivo; a cocktail of 3 differently targeted (anti-EGFR, anti-EpCAM and anti-HER2 IgG) SERS NPs and an untargeted (isotype IgG) control have been used for in vitro staining of human lung cancer cell lines. Through ratiometric analysis, where the intensity of the targeted signal is normalized to that of the untargeted SERS signal, the proportion of biomarker-bound SERS NPs is obtained and is proportional to the biomarker expression. The long-term goal is to use SERS-enabled bronchoscopy with topical application of the NPs as a minimally-invasive technique in high-risk lung cancer populations to evaluate suspicious lesions in situ, including those detected on screening CT scans.

9531-125, Session PSun

Monitoring the variation of blood glucose by diffuse reflection spectrophotometry

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Actually in biomedical research are developing non-invasive and painless method for the glucose monitoring techniques. Diabetic patients have to prick their finger for a drop of blood 4–5 times a day to check blood glucose levels, causing trauma and stress to obtain blood samples, factors affecting the real values of glucose. The objective of this study was to monitor the variation of glucose by spectrophotometry diffuse reflection after ingestion of a glucose solution of 75 mg / dL. The sample consists of 10 non-diabetic individuals, which were taken the measure with the spectrophotometer in the palm of the hand fasting, 30, 60 and 120 minutes of ingesting the solution, furthermore, the blood glucose was determined by absorption in order to obtain a reference value of the glucose. Concluding that there is a correlation between the variation of the glucose values obtained from diffuse reflectance spectrophotometer with the variation of blood glucose by the conventional method

9531-126, Session PSun

Photopolymerization method and device to treat aneurysms

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No Abstract Available.

9531-127, Session PSun

The ablation threshold of Er;Cr:YSGG laser radiation in bone tissue

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In laser'cut clinical applications, the use of energy densities lower than the ablation threshold causes increase of temperature of the irradiated tissue, which might result in an irreversible thermal damage. Hence, knowing the ablation threshold is crucial for insuring the safety of these procedures. The aim of this study was to determine the ablation threshold of the Er,Cr:YSGG laser in bone tissue.

Bone pieces from jaws of New Zealand rabbits were cut as blocks of 5 mm x 8 mm and polished with sandpaper. The Er,Cr:YSGG laser used in this study had wavelength of 2780 nm, 20 Hz of frequency, and the irradiation condition was chosen so as to simulate the irradiation during a surgical procedure. The laser irradiation was performed with 12 different values of laser energy densities, between 3 J/cm2 and 42 J/cm2, during 3 seconds, resulting in the overlap of 60 pulses. This process was repeated in each sample, for all laser energy densities.

After irradiation, the samples were analyzed by scanning electron microscope (SEM), and it was measured the crater diameter for each energy density. By fitting a curve that related the ablation threshold with the energy density and the corresponding diameter of ablation crater, it was possible to determine the ablation threshold. The results showed that the ablation threshold of the Er,Cr:YSGG in bone tissue was 1,95(42) J/cm2.

9531-128, Session PSun

Optical coherence tomography applied to the evaluation of wear of composite resin for posterior teeth

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Resin composites are widely used as restorative materials due to their excellent aesthetical and mechanical properties. Posterior teeth are constantly submitted to occlusal stress and upon restoration require more resistant resins. The aim of this study was to analyze in vitro the wear suffered over time by restorations in composite resins in posterior teeth, by Optical Coherence Tomography (OCT). 30 molars had occlusal cavities prepared and were randomly divided into three groups (n=10) and restored with composite resin: G1: Filtek P90 (3M/ESPE), G2: Tetric N-Ceram (Ivoclar Vivadent); G3: Filtek P60 (3M/ESPE). Specimens were subjected to initial analysis by OCT (OCP930SR, Thorlabs, axial resolution 6.2 ?m) and stereoscopic microscope. Specimens were submitted to thermocycling (500 cycles, 5-55 °C) and subjected to simulated wear through a machine chewing movements (Wear Machine WM001), projecting four years of use. After mechanical cycles, the specimens were submitted to a second evaluation by the OCT and stereoscopic microscopy. As a result, it was observed that 90% of the restorations of both groups had fractures and/or points of stress concentration, considered niches for early dissemination of new fracture lines. It was also found that G1 and G2 had more points of stress concentration, whereas G3 had a higher incidence of fracture lines already propagated. It was concluded that the G3 showed more brittle behavior at the masticatory wear when compared to G1 and G2.

9531-129, Session PSun

Adapting smartphones for low-cost optical medical imaging

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Optical images have been used in several medical situations to improve diagnosis of lesions or to monitor treatments. However, most systems employ expensive scientific (CCD or CMOS) cameras and need computers to display and save the images, usually resulting in a high final cost for the system. Additionally, this sort of apparatus operation usually becomes more complex, requiring more and more specialized technical knowledge from the operator. Currently, the number of people using smartphone-like devices with built-in high quality cameras is increasing, which might allow using such devices as an efficient, lower cost, portable imaging system for medical applications. Thus, we aim to develop methods of adaptation of those devices to optical medical imaging techniques, such as fluorescence. Particularly, smartphones covers were adapted to connect a smartphone-like device to wide-field fluorescence imaging systems. These systems were used to detect lesions in different tissues, such as cervix and mouth/throat mucosa, and to monitor ALA-induced protoporphyrin-IX formation for photodynamic treatment of cervical cancer. First results allowed monitoring protoporphyrin-IX effectively, and normal versus abnormal tissue differences were observed. This approach may contribute significantly to low-cost, portable and simple clinical optical imaging collection.

9531-130, Session PSun

Diagnosis of occlusal caries lesions in primary molars started in default for spreading of coherent light speckle

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The detection of incipient occlusal caries lesions is a difficult task which requires a rigorous examination is commonly used visual and radiographic inspection. Dental caries by inducing mineral loss alters the optical properties of the affected tissue, so the study of these properties may produce non-invasive and non-destructive methods for early diagnosis of caries. Thus, the goal of this project is to correlate the results obtained by visual examination and by ICDAS method for scattering pattern of coherent light speckle (grainy optical statistical patterns (speckle) generated by healthy and injured dental tissue) .For both are used 30 primary molars teeth collected from the bank of human teeth Universidade Nove July that will carious lesions induced by the method of pH cycling. The samples will be evaluated for making the diagnosis of caries using two methods: ICDAS and speckle in periods 5, 10 and 15 days of induction of caries after and the results will be statistically analyzed using a significance level of 95%.

9531-131, Session PSun

FT Raman spectroscopy in the study of human teeth under medications demineralization

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The in situ study evaluated antihistamine(DA) and bronchodilator(DB) drugs actions on dental enamel using FT Raman spectroscopy

Analysis of pH drugs were permorfed, DA 1.48 and DB 2.90. Enamel (n=24) were analysed by FT - Raman and randomly distributed in control group (CG) and experimental groups (GEA and GEB), specimens fixed in palatine appliances . In CG, dripped 20% sucrose (8Xday/3 min)/7 days. In GEA, 20% sucrose (8Xday/3 min) + drug (4Xday/3 min). In GEB , 20% sucrose (8Xday/3 min) B + drug (4Xday/3 min). FT- Raman analysis was performed again. Data analyzed by Student t test and ANOVA

Differences in peak intensity of carbonate (CO3) /phosphate (PO4) (p<0.0286) post challenge in GEB related to baseline. The GEB integrated area of inorganic post challenge (p<0.0349).

Organic loss in GEA, area (p<0.0228) and intensity (p<0. 0471) between moments and in GEB , area (p<0.0243) and intensity (p<0.00276). Comparing organic area and intensity difference observed post challenge GC(0,31±0,13) and GEB(0,54±0,13). Area difference between GEA(0,30±0,16) X GEB(0,54±0,13) and intensity difference GC(0,09172±0,04) and GEB(0,16±0,04) and GEA(0,091±0,05) X GEB(0,16±0,04). Significant integrated area CO3/PO4 loss for GC(0,12±0,01)X GEB(0,16±0,02) and GEB X GEA(0,13±0,02). For intensity differences between GC(0,08±0,01) and GEB(0,10±0,01), and the last one and GEA(0,08±0,01)

After administration of drugs, reduction in the amount of carbonate, organic and inorganic components were observed, denoting possible demineralization

9531-132, Session PSun

Evaluation of the molecular mechanisms of bacterial resistance in Pseudomonas aeruginosa by FTIR microspectroscopy

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Pseudomonas aeruginosa is a gram-negative bacterium belonging to the group of non -fermenting glucose associated with high mortality , morbidity, mortality and prevalence in hospital environments . Being found from respiratory equipment to antiseptic gels surgical center . This characteristic to adapt to different environments is due to changes at the molecular level where it is modulated and combined multiple resistance mechanisms ensuring adaptation and survival. Understand the different stages of adaptation of the organism based on the molecular and biochemical changes modulated by its genome by FTIR microspectroscopy can contribute to the search for new technologies to develop antimicrobial or bacteriostatic strategies that impact directly on the resident microbiota .

9531-133, Session PSun

Effects of infrared laser on the bone repair assessed by x-ray microtomography (μCT) and histomorphometry

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The bone fracture is an important public health problem. The lasertherapy is used to accelerate tissue healing. Regarding diagnosis, few methods are validated to appreciate and follow the evolution of bone microarchitecture. The aim of this study was to evaluate the effects of lasertherapy on bone repair with x-ray microtomography (μCT) and histomorphometry. A transverse rat tibia osteotomy with Kirchner wire and 2mm width polymeric spacer were used to induce a delay bone union repair. Twelve rats were divided into two groups: Control Group (CG): untreated; Laser Group (LG): treated with laser. Twelve sessions of treatment (808nm laser, 100mW, 2500mW/cm2, 125J/cm?, 50seconds) were performed. The μCT scanner parameters were: 100kV, 100μA; Al+Cu filter and 9.92μm resolution. The volume of interest (VOI) was located 300 sections above and below the central region of the fracture, totaling 601sections or 5.96mm. The softwares CT-Analyzer, NRecon and Mimics were used for 2D and 3D analysis. Histomorphometry was also performed. One-way ANOVA and Tukey were used for statistical analysis. The connectivity (Conn) showed significant increase for LG than CG (32371±20689 vs 17216±9467, p?0.05). There was no significant difference for bone volume (59±19mm3 vs 47± 8mm3) and histomorfometric data [LG and CG showed greater amount of cartilaginous (0.19±0.05% vs 0.11±0.09%) and fibrotic (0.21±0.12% vs 0.09±0.11%) tissues]. The presence of the cartilaginous and fibrotic tissues is a negative effect which may be related to non-absorption of polymeric spacer and light distribution in biological tissues. However, the positive effect was greater bone connectivity, indicating improvement in bone microarchitecture.

9531-134, Session PSun

New speckle analysis algorithm for flow visualization in optical coherence tomography images

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Optical Coherence Tomography (OCT) is a noninvasive technique capable of generating in vivo high-resolution images. However, OCT images are degraded by a granular and random noise called speckle. Nevertheless, such a noise may be used to gather information regarding the sample, as is exploited by techniques like Speckle Variance – OCT (SV-OCT), used to map regions of underlying blood flow on a sample. SV-OCT is widely used in the literature, but the variance calculation is computationally expensive. Therefore, we propose a new algorithm to employ speckle in identifying flow.

Our method is based on the evaluation of intensity fluctuation between two consecutively acquired OCT images. It is expected that speckle arising from regions of flow present greater fluctuations in intensity over time, compared to speckle originating from static regions. Our proposal is to verify those fluctuations in many small windows of time, instead of calculating the variance over a single large window, improving processing time.

Our results were compared to those obtained by traditional method of Speckle Variance to demonstrate the feasibility of the technique. Both algorithms were applied to series of OCT images from a microchannel flow phantom, as well as from a biological tissue with blood flow. The results obtained by our method are in good agreement with those

from SV-OCT. We've also analyzed the performance of both algorithms, registering the processing time and memory use. Our method performed 31% faster with the same use of memory. Therefore, we demonstrated a new method to map flow on OCT images.

9531-135, Session PSun

New speckle analysis method for optical coherence tomography signal based on autocorrelation

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Optical Coherence Tomography (OCT) is a noninvasive imaging technique with high resolution widely used for in vivo applications. Nonetheless, OCT is prone to speckle, a granular noise that degrades the OCT signal. Speckle statistics may, nevertheless, reveal information regarding the scatterers from which it originates. This fact is exploited by techniques such as Speckle Variance-OCT (SVOCT). SVOCT, however, doesn't provide quantitative information, which is a major drawback for the use of speckle based techniques on OCT. In the present work we attack this problem, proposing a new method for analysis of speckle in OCT signal, based on autocorrelation. We associate the changes in decorrelation time of the signal with the changes in flow velocity. It is expected that greater velocities result in lower decorrelation times.

To verify that, milk was pumped through a microchannel at different velocities, and the decorrelation time was computed for a single point in the center of the microchannel, sampled at 8 kHz rate.

Our results suggest that for flows velocities greater than 0,83 mm/s it is possible to associate decorrelation time with flow velocity, while velocities below 0,42 mm/s are not distinguishable, supposedly due to the Brownian motion. For velocities above 5 mm/s our acquisition rate doesn't get enough sampling information, as the decorrelation time gets too low. These results indicate that Speckle based techniques may be used to get quantitative information of flow in OCT samples, which can be used to assist in many diagnostics modalities, as well as map such flow regions.

9531-136, Session PSun

Determination of radiation levels without producing damage to blood cells

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The blood components are exposed to substances that can be cause irreparable damage in this structure, altering biological and biochemical functions. Genetic factors or the improper consumption of chemicals, alcohol, drugs, and others, its very prejudicial to the health of any person; however, none of these cases produces so severe disorders such as radiation. From a practical point of view, this work investigated the effects contracts to radiate for a certain time, the platelet population of the blood system in humans (age ranging from 18 years to 35 years), with low-power lasers with wavelengths 632 nm (red light) and 532nm (green light).

The samples were exposed to both radiations, leaving one of the male and female samples without radiation, to serve as the control group to make quantitative comparisons. The exposure to 30 minutes with the green laser radiation of 532 nm, resulting in an increase of the 0.5% in the platelets population on a male sample and an increase of the 4.5% in the platelets population on a female sample, and with an exposure time of 60 minutes was obtained as a result an increase of the 3.5% in the platelets population on a male sample and an increase of the 1.4% in the platelets population on a female sample. In the experiment with red laser of 632nm, exposing the samples in an interval of 30 minutes, the results were an increase of the 7.9% in the platelets population on a male sample, and radiating to 60 minutes, was obtained an increase of the 0.7% in the platelets population on a male sample and the 5.5% on a female sample.

9531-137, Session PSun

Healing of burns with synthetic membrane and low intensity laser therapy

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Works conducted in experimental animals have shown that Low Intensity Laser Therapy (LILT) and the healing membranes are promising to modulate wound healing and prevent wound infection, respectively. However, the combination of both therapies for scald burns has not been studied. Thus, treatment of third degree burn was studied in an animal model, using synthetic membrane (Biocel NP®, Brazil) associated with LILT. 20 Wistar animals were divided into five groups: GC - burn was not subjected to treatment; GM - burn was subjected to treatment with the membrane; GL - burn was subjected to LILT; GML - burn was subjected to treatment with the membrane and LILT; GS - burn was subjected to conventional treatment with 1% silver sulfadiazine. At the end of 7, 14, 21 and 28 days after establishment of burns, the animals were euthanized and lesions removed for histological preparation. The presences of inflammatory infiltrate, granulation tissue and ulcer were analyzed. The ordering of type I collagen was also measured. According to the results, two statistical tests were used: the two-sample Poisson rate and Kruskal-Wallis test for analysis between groups, with a significance level of 95%. On the 28th day there was a significant difference between groups silver sulfadiazine and membrane + LILT with a frequency of inflammatory cells two times lower in the latter group. There is indication that GML excelled from 21th to 28th days, making it a beneficial therapy for remodeling phase, which could also be checked by organization of collagen.

9531-139, Session PSun

Comparative analysis of gingival phenotype in animal and human experimental models using optical coherence tomography in a non-invasive approach

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Imaging methods are widely used in diagnostic and among the diversity of modalities, optical coherence tomography (OCT) is nowadays commercially available and considered the most innovative technique used for imaging applications, in both medical and non-medical applications. In this study, we exploit the OCT technique in the oral cavity for identification and differentiation between free and attached gingiva, as well as determining the gingival phenotype, an important factor to determination of periodontal prognosis in patients. For the animal studies, five porcine jaws were analyzed using a Spectral Domain SD-OCT system operating at 1325nm and stereomicroscope, as gold pattern. The SD-OCT at 1325nm was chosen due to the longer central wavelength, that allows to deeper penetration imaging, and the faster image acquisition, an essential factor for clinical setting. For the patient studies, a total of 30 males and female were examined using the SD-OCT at 1325nm and computer controlled periodontal probing. 2D and 3D images of tooth/gingiva interface were performed, and quantitative measurements of the gingival sulcus could be noninvasively obtained. Through the image analysis of the animals jaws, it was possible to quantify the free gingiva and the attached gingiva, the calculus deposition over teeth surface and also the subgingival calculus. For the patient's studies, we demonstrated that the gingival phenotype could be measured without the periodontal probe introduction at the gingival sulcus, confirming that OCT can be potentially useful in clinic for direct observation and quantification of gingival phenotype in a non-invasive approach.

9531-141, Session PSun

Enhancing the optical contrast in tissue slide analysis using Red, Green and Blue LEDs as microscope light source

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Optical microscope usually has a white lamp as light source. The tissue slide samples usually are stained with HE (hematoxylin and eosin) to improve the visualization of cell structures. To improve the image contrast, there is the possibility of adding filters that alter the color of light being projected onto the sample. However, there are limitations in filters use, there is no variation in the added color. In addition, with this method it is not possible to overlap the filters in order to obtain other colors. An alternative to improve the tissue slide analysis is the use of Red, Green and Blue (RGB) LEDs as light source, allowing the variation of the intensity of each channel, and creating any light color. In this study we assembled a homemade RGB LED device to be attached in a conventional optical microscope. The images were taken using a microscope (Eclispe TS, Nikon, Japan), where the original light source was easily replaced by a new one. All the images were taken from the same slide spot with both light sources and a comparison performed. For each tissue type, a different RGB proportional is proposed and allowed an improvement in the visual contrast.

9531-142, Session PSun

In situ visualization of dermal collagen dynamics during skin burn healing using second-harmonic-generation microscopy

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Burn healing is a process to repair thermally damaged tissues. Although burn healing has many aspects, it is common for dynamics of collagen fiber, such as decomposition, production, or growth, to be closely related with burn healing. If such healing process can be visualized from the viewpoint of the collagen dynamics, one may obtain new findings regarding biological repairing mechanisms in the healing process.

Second-harmonic-generation (SHG) light is an effective optical probe because of high selectivity and good image contrast to collagen molecules as well as high spatial resolution, optical three-dimensional (3D) sectioning, minimal invasiveness, deep penetration, the absence of interference from background light, and in vivo measurement without additional staining. Furthermore, since SHG light arises from a non-centrosymmetric triple helix of three polypeptide chains in the collagen molecule, its intensity decreases and finally disappears when thermal denaturation caused by the skin burn changes the structure of this molecule to a centrosymmetric random coil. Therefore, optical assessment of skin burn has been investigated by SHG microscopy. In this paper, we applied SHG microscopy for in situ imaging of the healing process in animal skin burn and successfully visualized the decomposition, production, and growth of renewal collagen fibers as a series of time-lapse images in the same subject.

9531-143, Session PSun

Optical fluorescence spectroscopy to detect hepatic necrosis after normothermic ischemia: animal model

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Liver transplantation is a well-established treatment for liver failure. However, the success of the transplant depends on liver graft condition. The tissue function evaluation during the several transplantation stages is relevant, in particular during the organ harvesting, when a decision is made concerning the viability of the graft. Optical fluorescence spectroscopy is a good option because is a non-invasive and fast technique. A partial normothermic hepatic ischemia was performed in rat livers, with a vascular occlusion of both median and left lateral lobes, allowing circulation only for the right lateral lobe and the caudate lobe. Fluorescence spectra under excitation at 532 nm (doubled frequency Nd:YAG laser) were collected using a portable spectrometer (USB2000, Ocean Optics, USA). The fluorescence emission was collected before vascular occlusion, after ischemia, and 24 hours after reperfusion. A morphometric histology analysis was performed as the gold standard evaluation ? biopsy samples were analyzed, and the percentage of necrotic tissue compared to viable liver tissue was obtained. The results showed that changes in the fluorescence emission after ischemia can be correlated with the amount of necrosis evaluated by a morphometric analysis. The laser-induced fluorescence spectroscopy technique after normothermic ischemia showed to be a fast and efficient method to differentiate ischemic injury from viable tissues.

9531-144, Session PSun

CdTe QUANTUM DOTS-TRANSFERRIN BIOCONJUGATES AS FLUORESCENT PROBES FOR CANCER CELLS

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Quantum dots (QDs) are novel fluorescent nanomaterials with excellent optical properties, including broad absorption bands and high photostability, which have been widely used for cell, tissue and in vivo imaging. Some cancer cells have a high metabolic rate and, therefore, can show over expression of receptors at their surface, including the Transferrin receptor. The aim of this work was to conjugate CdTe QDs to Holo Human Transferrin (Tf) and apply these bioconjugates for labeling purposes and for studying their interactions with cancer cells. QDs were synthesized in aqueous medium using Te2- and CdCl2 as precursors and mercaptosuccinic acid (MSA) as a stabilizing and functionalizing agent. After synthesis, QDs were covalently bound to Tf using EDC and Sulfo-NHS as coupling agents. The bioconjugation process was characterized by emission and absorption spectroscopy and confirmed by electrophoresis and fluorescence microplate assay (FMA). Then, bioconjugates were applied to cervical cancer (HeLa) cells, which were analyzed by fluorescence confocal microscopy. FMA results showed that QDs-Tf conjugates presented a signal of 5063% higher intensity than controls, indicating that the conjugation was efficient. The electrophoresis study confirmed this result. According to fluorescence confocal microscopy, only QDs-Tf, and not bare QDs, interacted with the Tf-receptors present in at the surface of HeLa cells. The specificity of labeling was confirmed by a Tf-receptor inhibition assay, using Tf (10 mg/mL) before incubation with

QDs-Tf. The overall results show that QDs-Tf conjugates, developed using an efficient bioconjugation protocol, resulted in a specific and sensitive fluorescent probe which may be applied to help understanding of cancer biology.

9531-145, Session PSun

Simplified variant of an optical chip to evaluate aggregation of red blood cells

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In the last decades, the evolution of knowledge in Hemorheology has made this branch of science an indispensable tool for the evaluation of specific clinical situations, particularly those related to vascular pathologies. Specific experimental conditions and the definition of parameters in a rheological approach to blood and blood components lead to the design of new equipment and techniques generally founded on optical and mechanical principles. Traditional techniques that evaluate the aggregation of red blood cells by optical methods require large sample amount and provide parameters that vary significantly from one method to another. In addition, the instruments used are usually costly, unwieldy and bulky, so these hemorheological measurements are not usually performed in hospitals, although they provide important information for the benefit of human health. A simplified variant of the chip system developed by Shin et al. (Clin Hemorheology Microcirculation 41:197-207, 2009) based on light transmission for measuring the kinetics of erythrocyte aggregation is presented. Through a detailed analysis of intensity versus time curves, relevant information of the erythrocyte aggregation phenomenon and its variables (hematocrit, erythrocyte surface charge, plasma protein content, etc.) is obtained. New parameters that provide more accurate for the diagnosis and prevention of microcirculatory disturbances in order to have an immediate application in Clinical Medicine are proposed.

9531-146, Session PSun

Nonlinear ellipse rotation method for biophotonics images applications

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The use of nonlinear optical processes in microscopy has brought considerable advances to obtain three-dimensional images. Several areas like biology look for images with better resolving power, less invasive and contrasts, for instance. Each nonlinear effect gives different image contrast, making possible to obtain information about the interfaces, such as with third-harmonic generation or the volume, with two-photon absorption (2PA) induced fluorescence, for instance. In fact, the 2PA induced fluorescence is one of the most explored processes due to high sensitivity and simplicity. Nevertheless, sometimes there are drawbacks due to necessity of addition of external fluorescent chromophore, which can be poisonous for some biological sample. Recently, we developed a new sensitive method to measure with high precision nonlinear ellipse rotation (NER), which here, it could be used to nonlinear microscopy. Similar to 2PA induced fluorescence, the NER effect is capable of see the volume of the sample with no addition of any chromophore. In this case, the image contrast is given by the local nonlinear refractive index. In fact, this method gives the absolute value of the local nonlinearity. Here we did NER measurements with 150 fs laser pulses at 775 nm from a 1 kHz repetition rate amplified Ti:sapphire laser system. Preliminary studies with images of onion cells demonstrate the potential of this new method.

9531-147, Session PSun

Fluorescence imaging system for detection of Propionibacterium acnes

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Acne is the most common skin disease found by dermatologists, where it is considered chronic because it has a high degree of recurrence may affect 50% of the population in adult life. Several studies have shown good results for treatment of acne via phototherapy, however, some studies emphasize that this type of treatment is still new and additional and more detailed studies should be conducted. This paper presents the development and validation of equipment based on fluorescence images, for monitoring Protoporphyrin IX (PpIX), produced by Propionibacterium Acnes, who is primarily responsible for the cause of Acne, such as its use in monitoring formation, accumulation and photobleaching of PpIX during PDT applied to Acne. The equipment developed consists of an optical system, mechanical, electronic and detection. The optical part consists of a high intensity LED (405 nm) and 5 optical components: 1 - bandpass filter, 2 - notch filter, 3 - longpass filter, 4 - dichroic beamsplitter, 5 - anti-reflection filter and a biconvex lens. Four distinct experiments were performed in order to validate the detection of bacteria present on the face or scalp of volunteers. It is too early a conclusive analysis of the efficiency of detection of fungi and bacteria and its correlation with the cause of dermatitis and acne, however, initial tests point to this direction. The prototype shows good intensity and uniformity of illumination and good quality images for the detection of fluorescence in the red area, provided by the choice and optimization of optical filters used.

9531-148, Session PSun

Comparison between two portable devices for widefield PpIX fluorescence during Cervical Intraepithelial Neoplasia treatment

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The use of portable electronic devices, in particular mobile phones such as smartphones is increasing not only for all known applications, but also for diagnosis of diseases and monitoring treatments like topical Photodynamic Therapy. The aim of the study is to evaluate the production of the photosensitizer Protoporphyrin IX (PpIX) after cream containing methyl aminolevulinate (MAL) in the cervix with diagnosis of Cervical Intraepithelial Neoplasia (CIN) through the fluorescence images captured after one and three hours and compare the images using two devices (a Sony Xperia® mobile and an Apple Ipod®). Was observed an increasing fluorescence intensity of the cervix three hours after cream application, with in both portable electronic devices. However, because was used a specific program for the treatment of images usingthe Ipod® device, these images presented better resolution than observed by the Sony cell phone without a specific program. One hour after cream application presented a more selective fluorescence than the group of three hours. In conclusion, the use of portable devices to obtain images of PpIX fluorescence shown to be an effective tool and is necessary the improvement of programs for achievement of better results.

9531-149, Session PSun

Validation of a new real-time in-situ optical coherence tomography with modified oral probe by comparing with the certified CE marking optical coherence tomography dermatology probe

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A non-standard, non-CE certified recently modified optical coherence tomography (OCT) oral instrument, was calibrated and validated against the same manufacturer's standard commercial CE certified OCT skin instrument. Comparison between both instruments was achieved by measuring National Physical Laboratory (NPL) optical dimensional standards and metric slip gauges in X, Y, and Z-axes,?i.e. 3-D object measurement. Further, calibration of step height model (Z axis) and planar slope phantoms, crossing from air to aqueous media, were compared to investigate relative image behaviour across changing refractive index environments.

Results of the comparisons demonstrated that the new OCT oral system performed in a similar fashion to the CE certified OCT skin instrument and can be used as a surrogate in measurement of cutaneous and oral tumour thickness and depth with confidence and accuracy.

9531-150, Session PSun

Identification of atherosclerosis using aminolevulinic gold nanoparticle assay in faecal specimens

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Atherosclerosis is a chronic degenerative disease that affects medium and large caliber arteries, and is characterized by lesions with plaque or atheroma. The atheromatous plaques exhibit an accumulation of protoporphyrin IX, or PPIX, which is transferred to the blood and feces. In this work it was associated a precursor of the PPIX, the 5-aminolevulinic acid, ALA, with gold nanoparticles (ALA:AuNps). The objective was verify the possibility to use ALA:AuNPs as a diagnosis agent for atherosclerosis. ALA:AuNps were synthesized mixing ALA with Tetrachloroauric(III) acid in miliQ water solution followed by photoreduction with light from a Xenon lamp. The synthesized nanoparticles were characterized by UV/Vis optical absorption, Zeta Potencial and electron microscopy. A total of 12 Male New Zealand rabbits were divided into 3 groups: control group (CG) where animals received normal diet, control group with ALA (CGALA) and Experimental Group with ALA:AuNPs (EGALAAu) in which the animals received a diet with 1% cholesterol. Changes in the animal's arteries throughout the study were analyzed by optical microscopy techniques. Animal feces were collected after 34 days of diet introduction, and porphyrins were extracted and analyzed. Measurements of the emission intensity of extracted porphyrins from the feces in the region between 575 and 725 nm were done. An increase in the feces porphyrin emission after ALA:AuNPs administration suggests that ALA was incorporated by the gold nanoparticles, and its structure remained unchangeable and a rapid conversion into endogenous porphyrins, overloads the synthetic pathway and leads to the accumulation of PpIX. This finding indicated that this method can aid in the early diagnosis of atherosclerosis with high sensitivity.

9531-151, Session PSun

Characterization of probe contact effects on diffuse reflectance spectroscopy measurements

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Diffuse Reflectance Spectroscopy (DRS) is a rapid, non-invasive optical method widely adopted to gain diagnostic information of human tissue. The most flexible approach to this method is a fiber-optic contact-probe used with a portable spectroscopy system. A challenge of this method is that the external pressure brought by the contact probe can significantly affect the tissue optical properties as well as the light coupling into the probe, and thus influence the collected DRS-spectrum.

By combining commercially available VIS- and NIR-spectrometers, we have access to the full wavelength range from around 400 to 1700 nm. Using this system together with various fiber-optic contact-probes, we have acquired DRS-spectra from human skin tissue in-vivo under well-controlled indentation to study the effects of probe contact pressure on DRS-measurements. For assessing the tissue biomechanical behavior, a finite element model (FEM) have been employed, whereas the Monte Carlo (MC) method as well as a diffusion theory model (DT) have been used to simulate the DRS-spectrum of the tissue under indentation. The focus in this work is on the effect of probe contact pressure on the intrinsic tissue biomechanical and optical properties. The pressure was varied by a calibrated loaded-spring system used with a fiber optic probe. The goal is to find a practical method that solves the issue of probe contact effects in clinical situations.

9531-152, Session PSun

UV protection of Euglenids: computation of the electromagnetic response

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Euglenids are a group of predominantly free-living unicellular microorganisms that mostly live in freshwater bodies but can also be found in marine and brackish waters. These organisms have a characteristic that distinguishes them form the other protists: they are covered by a surface pellicle formed by S-shaped overlapping bands which resemble a diffraction grating.

Euglenids have developed numerous protection mechanisms intended to avoid or reduce the damage produced by UV radiation, such as the production of pigments and the repair mechanisms in hours of darkness and during daylight. In a recent paper we have investigated the role played by the pellicle of Euglenids in the protection of the cell against UV radiation, by means of an electromagnetic approach based on the approximation of the pellicle profile by a one-dimensional diffraction grating. This simplified model allowed us to confirm that under certain incidence conditions, the corrugation of the pellicle helps increase the UV reflection, and consequently, diminish the UV radiation that enters the cell. In order to further extend our conclusions, in this work we study the behavior of the whole cell under optical and UV illumination. We apply two different approaches to calculate the reflected response: a simulation method especially developed to deal with complex biological structures that permits the introduction of the scattering object via an electron microscopy image, and the integral method, which has been widely used to compute the electromagnetic response of finite structures. We show the results obtained and analyze their applicability as artificial UV reflectors.

9531-153, Session PSun

Gastrointestinal tract disease diagnosis by hyperspectral endoscope

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We have developed a prototype endoscope system integrating wide-field structure imaging and reflectance-based hyperspectral imaging modalities and the study was used to proof applicability of hyperspectral imaging of human gastrointestinal mucosal surfaces in vivo. A hyperspectral endoscopy system was built and data cubes at visible region (400-630nm) were collected. The system is used to successfully acquire the oxyhemoglobin spectral of a region consisting of blood capillaries.Then Sensitive wavelengths relating to physiological signature or disease was found by the analysis of principle component analysis.

9531-154, Session PSun

Time-resolved and steady-state fluorescence spectroscopy for the assessment of skin photoaging process

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Intrinsic fluorescence from biological tissue can be exploited for basic studies of physiology and pathology.1 The optical properties of these intrinsic fluorophores respond to the microenvironment and the metabolic status, thus making fluorescence spectroscopy a valuable tool to study the conditions of biological tissues.2 The purpose of this study is to investigate the hairless mice skin metabolic changes during the photoaging process through lifetime and fluorescence measurements targeting NADH and FAD. Excitation of NADH and FAD is performed by two lasers centered at 378 nm and 445 nm, respectively. To investigate different skin depths, two optical probes are used for delivering and collecting the light. The fluorescence acquisition is carried out at mice dorsal and ventral regions throughout the photoaging protocol and aging process. Differences in fluorescence and lifetime data between young and photoaged mice measurements were observed. The autofluorescence spectrum of photoaged flank skin showed an increase compared to young and aged skin. For dorsal photoaged skin, a slightly increase of free NADH and bound FAD quantities and a slightly decrease of free and bound NADH and FAD lifetimes using the depth investigation probe were observed comparing to the normal one. The photoaging process investigated with the normal probe showed the most pronounced change in the bound NADH lifetime, which constantly increased from week one until the end of the protocol. Measurements with the depth probe are in progress. Aging process is being investigated to complement the information obtained from fluorescence data and lifetime of photoaging process.

9531-155, Session PSun

Study of Lumineers interfaces by means of optical coherence tomography

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Lumineers, which are ceramic laminates used for esthetical restorations, were evaluated with optical coherence tomography (spectral domain SD-OCT, 930nm central wavelength). OCT has been used to evaluate dental materials, and is employed here to evaluate lumineers for the first time. Lumineers are used as indirect restoration, and after wearing and aging, several undesirable features such as gaps, microfractures and cracks can appear in which would only be seen by invasive analysis. In this work, we employed 2D and 3D OCT images to evaluate noninvasively those materials, as well as the cement-laminate interface. We analyzed two resin cements (RelyX Veneer, 3M ESPE, and Variolink® Veneer, Ivoclear Vivadent), and two recommended adhesives by selected manufacturers: Single Bond 2 and Excite F, employing two different cementation protocols. Initial measurements showed that the SD-OCT was able to see through the 500?m thick lumineer, as delivered by the fabricant, and no failure was observed. After cementation on 20 different bovine tooth samples, which will go through a thermo cycling process to simulate thermal stress in a oral cavity, the samples will be re-evaluated using the OCT system. The expected results shall demonstrate the feasibility of using OCT as a noninvasive technique for evaluation of the lumineers after mimicking oral use, opening up the possibilities of clinical use.

9531-156, Session PSun

Diffuse reflectance imaging to predict heterogeneities in turbid optical phantom

Thereza C. Fortunato, Cristina Kurachi D.D.S., Vanderlei S. Bagnato, Lilian T. Moriyama, Univ. de São Paulo (Brazil)

The use of light as a therapeutic agent has been the subject of several studies; however, the dosimetry for its clinical application is still based on empirical data. The propagation of light in biological tissues depends on the tissue optical properties, and these properties may vary among individual, tissues and sites, making difficult the establishment of a dosimetry. In this context, it becomes essential to seek for the development of methods capable of determining the spatial distribution of light in biological tissues individually, allowing also the individualization dosimetry. This study aims to image the diffuse reflectance at the optical phantom surface to infer the spatial distribution of light inside the phantom when and absorbing obstacle is present. Red and Near-infrared lasers were used as light source on liquid and solid turbid optical phantom; a small black sphere was used as absorbing obstacle. It is important to evaluate in real time and in a non-invasive way the existence of heterogeneities that may compromise the light propagation inside a biological tissue, so that the light dosimetry might be properly established.

9531-157, Session PSun

Portable fluorescence microendoscope system for smartphones and imaging processing software

Pablo A. Gómez García, Cristina Kurachi D.D.S., Sebastião Pratavieira, Univ. de São Paulo (Brazil)

A portable microscope/microendoscope will be presented. The system was specially designed for smartphones and taking into account its simplicity, will be able to bring this technology to almost every doctor's office. It is worth mentioning its flexibility of use, that allows several modes since all the components are interchangeable (the illumination LED, the lenses, the dichroic mirror and the filters) which permits different applications and also its economic price, that does not exceed US$ 1000. The system can be used as a conventional microscope or as a microendoscope with a fiber bundle with different LED for illumination depending on the sample. Its first practical application will be to examine cervix tissue (invivo and exvivo) using Acriflavine as dye, but different applications are under evaluation and will be carried out on the future. Also a software to process the images that obtain the important parameters has been develop in order to determine altered tissue creating a complete diagnostic technology.

9531-158, Session PSun

Onycomicosis diagnosis using fluorescence and infrared imaging systems

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Onychomycosis is a common disease of the nail plate, constituting approximately half of all cases of nail infection. Onychomycosis diagnosis is challenging because it is hard to distinguish from other diseases of the nail lamina such as psoriasis, lichen ruber or eczematous nails. The existing methods of diagnostics so far consist of clinical and laboratory analysis, such as: Direct Mycological examination and culture, PCR and histopathology with PAS staining. However, they all share certain disadvantages in terms of sensitivity and specificity, time delay, or cost.

This study aimed to evaluate the use of infrared and fluorescence imaging as new non-invasive diagnostic tools in patients with suspected onychomycosis, and compare them with established techniques.

For fluorescence analysis, a Clinical Evince (MM Optics®)was used, which consists of an optical assembly with UV LED light source wavelength 400mm ± 10nm and the maximum light intensity: 40 mW / cm? ± 20%. For infrared analysis, a Fluke® Camera FKL model Ti400 was used. Patients with onychomycosis and control patients without group lesion were analyzed. The fluorescence images were processed using MATLAB® routines, and infrared images were analyzed using the SmartView® 3.6 software analysis provided by teh company Fluke®.

The results demonstrated that both infrared and fluorescence can be complementary to diagnose different types of onychomycosis lesions. The simplicity of operation, quick response and non-invasive assessment of the nail patients in real time, are important factors to consider to ensure the implementation of this technology as a treatment for this common infection.

9531-159, Session PSun

Fluorescence for Optimization of Skin Cancer PDT Treatment

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The photodynamic therapy (PDT) is an alternative technique indicated for superficial basal cell carcinoma (BCC). The objective of this study is to present the importance of fluorescence monitoring for PDT. The lesion well prepared to PDT is confirmed by photosensitizer presence and its good distribution. With the aid of the fluorescence of photosensitizer the PDT effectiveness may improve and the cancer skin recurrence should be lower. Our proposition is the use of wide field fluorescence imaging to evaluate the PDT protocol in situ and in real time lesion of basal cell carcinoma. This evaluation procedure is performed with the monitoring of the production of protoporphyrin IX (PpIX) induced by methyl aminolevulinate, and with the detection of PpIX photobleaching during and after illumination. The fluorescence images provide information correlated with distinct clinical features and with the treatment outcome. The BCC lesions are presented and discussed in this work. Different fluorescence patterns of PpIX production and photobleaching could be correlated with the treatment response. The presented results show the potential of using wide field fluorescence imaging as a guidance tool to PDT.

9531-160, Session PSun

Preparation and optimization of aminolevulinic acid with gold nanoparticles for phototermal and photodynamic therapies applications

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ALA is a naturally occurring compound, the early intermediate in the heme biosynthesis pathway. When ALA is administered externally, the abundantly produced protoporphyrin IX (PpIX) cannot be quickly converted to heme, and therefore accumulates within cells. When activated by light, in a process known as photodynamic therapy, or PDT, this PpIX accumulated in the target cells can produce cytotoxicity. The use of gold nanoparticles (AuNps) as the vehicle for 5-Aminolevulinic acid (ALA) delivery is a promising approach, especially with the recent demonstration that immobilization of photosensitizer on the particle surface is better for reactive oxygen species (ROS) formation increasing cytotoxicity. In this study spherical ALA:AuNps were synthesized mixing ALA with Tetrachloroauric(III) acid in milliQ water solution followed by photoreduction by light from a Xenon lamp. An increase in the PpIX blood fluorescence was observed after oral administration of spherical ALA:AuNps in rabbits. This result demonstrated that ALA:AuNps participate of heme biosynthesis. The disadvantage of using spherical ALA:AuNps as agents for photonics therapies (PDT or photothermal therapy - PTT) is that their absorption band, around 540 nm, overlaps with the skin absorption, limiting the penetration depth of the excitation light. However, gold nanorods (AuNRs) present an absorption spectrum shifted to 700 - 900 nm, within the skin transparency window, which allows excitation of the nanoparticles situated deeper in the tissues. Here, we describe a new synthesis method that was applied to control the shape of the gold nanoparticles during its synthesis. To obtain ALA:AuNRs, precursor ALA:AuNps were irradiated by ultrashort laser pulses. The variation of the laser parameters such as pulse energy and duration, irradiation time and repetition rate was assessed. The relevant mechanisms contributing for the AuNRs production are discussed. The formation of ALA gold nanorods (ALA:AuNRs) was confirmed by visible-IR absorption spectroscopy and TEM microscopy.

9531-161, Session PSun

Development of automated prototype for studying the effect of solar aging on sunglasses

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The first Brazilian standard for UV protection sunglasses, NBR15111, was drafted and published in 2003, hitherto a faithful copy (mirror) of European, BSEN1836 standard. From 2010 to 2013, the School of Engineering of São Carlos (USP) made contribution in the review and drafting of this standard and the main change so far is on the extension of the UV range analysis for protection of sunglasses, i.e. from 280 - 380nm to 280-400nm. In previous studies, there are indications that ultraviolet protection degrades with use and exposure of sunglasses to natural ultraviolet radiation. Thus, this project aims to build a prototype for irradiating sunglasses lenses, where one of the spectacles will be submitted to the solar simulator; and the other to the prototype. This prototype consists of a panel with cover, which houses 100 lenses arranged in the use position and which will be irradiated by the sun from sunrise until sunset. The lid opens automatically and should turn towards the sun, so that the lens will always be irradiated from the front by the sun. Sensors will be installed to close the cover and protect the glasses of undesirable weather conditions and to determine the ultraviolet index to which the lenses are being subjected to. The exposure time and UV index will be recorded and automatic opening or closing the lid may also be interfered by a PC by online software. Previously to irradiation,

spectroscopy will be performed and then repeated after every 30 days of exposure.

9531-162, Session PSun

Diffuse reflectance spectroscopy of liver tissue

Nina Reistad, Stefan Andersson-Engels, Jan Nilsson, Christian Sturesson, Oskar Vilhelmsson Timmermand, Lund Univ. (Sweden)

The current clinical rationale for resection of colorectal liver metastases is often to administer chemotherapeutics before liver resection. The advantage of this approach is that 15-50% of patients, having initially irresectable liver metastases, can undergo surgical resection after the course of chemotherapy due to tumour shrinkage. However, the chemotherapeutic drugs can also induce liver damages such as steatosis, steatohepatitis (CASH) and sinusoidal obstruction syndrome (SOS). These damages can cause post-resectional liver failure. Hence, there is a need for intraoperative quantification and staging of liver damage induced by preoperative chemotherapy that can assess the physiological state of the liver before liver resection.

Diffuse reflectance spectroscopy (DRS) with a fiber-optic contact probe is a cost-effective, rapid, and non-invasive optical method widely adopted to extract diagnosis information of human tissue. By combining commercially available VIS- and NIR-spectrometers with various fiber-optic contact-probes, we have access to the full wavelength range from around 400 to 1700 nm. Using this flexible and portable spectroscopy system, we have acquired ex-vivo DRS-spectra from murine, porcine, and human liver tissue. For extracting the tissue optical properties from the measured spectra, we have employed the Monte Carlo (MC) method as well as a diffusion theory model (DT).

The focus in this work is on the capacity of this DRS-technique in discriminating metastatic tumor tissue from normal liver tissue as well as in assessing and characterizing damage to non-malignant liver tissue induced by preoperative chemotherapy for colorectal liver metastatic.

9531-163, Session PSun

OCT imaging for non-invasive assessment of hemangioma vascular lesions in children

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Hemangiomas are vascular tumors of the dermis capillaries that often involve the head, neck and oral cavity, with light to severe disfigurement. Early diagnosis and recognition of the lesion is essential to provide appropriate treatment and decrease harmful cosmetic and psychological effects. Critical parameters for diagnosis are the presence of caverns, the density, depth and diameter of blood vessels as well as the intensity of their blood flow. Excisional biopsies are the gold-standard but may cause severe bleedings and side-effects, so that a non-invasive imaging technique should be preferred.

We propose to use Optical Coherence Tomography (OCT), a morphological in-vivo optical imaging technique, coupled to functional blood-flow Doppler modality. We imaged hemangiomas on child patients of the IMIP hospital from less-than-one to seven year-olds. The OCT system is a Thorlabs swept source OCT at a wavelength of 1325 nm. The system has a frame rate of 25 images per second, axial and transverse resolution in tissue of 9 μm and 18 μm respectively, maximum detection depth in skin of approximately 1 mm.

We were able to distinguish between normal skin and vascular lesion areas. The OCT images of healthy skin show the different skin layers: the stratum corneum, epidermis and dermis, without special structures and vessels. On the contrary, the images of the lesion reveal empty structures that can be vessels or cavernous structures. The use of the Doppler effect allows to extract the blood flow information within the vessels and distinguish between blood vessels and empty cavernous structures. Although quantitative value of blood flow was not assessed, we can qualitatively report a high variability of blood flow intensities.

Our findings in terms of density, size and depths of blood vessels are consistent with previous literature on the subject. The high standard-deviation of our statistics can be explained by the high variability of the lesions as well as variability of vascularity inside one lesion. We are currently enrolling more patients in the study to reach a total of 50 patients and increase statistical significance.

We have shown that OCT completed by Doppler OCT is a promising method for non-invasive diagnosis and monitoring of vascular lesions.

9531-164, Session PSun

Fluorescence multi-scale endoscopy and its applications in the study and diagnosis of gastro-intestinal diseases

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Endoscopy is a reference technique frequently used in the diagnosis of several gastro-intestinal pathologies as Crohn disease, ulcerative colitis or colorectal cancer. It has a great potential as a detection technique of precocious alterations in the intestinal mucosa, which can lead to inflammatory processes or neoplams. However, this early lesions usually can't be detected with conventional endoscopes, due to lack ofcellular detail and the absence of specific markers. Because of that the development of new endoscopy technologies which are able to show microscopical changes in the mucosa structure are necessary. That is the case of the confocal endomicroscopy which in combination with a fluorescence endoscope offers fast and specific macroscopic information and a detailed analysis at celular level of the possible altered tissue areas.

To address the above-mentioned issue, a multimodal imaging module, compatible with comercial endoscopes has been developed. This module will combine near-infrared fluorescence measurements (enabling specific imaging of markers of disesase and prognosis) and confocal endomicroscopy providing information at the cellular level. The multiscale endoscope will be used in animal models exhibiting gastro-intestinal diseases in order to analyze potential diagnostic markers.

9531-165, Session PSun

Laser speckle contrast imaging of blood flow from anesthetized mice: correcting drifts in measurements due to breathing movements

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Background: Laser speckle contrast imaging allows non-invasive assessment of cutaneous blood flow. Although the technique is attractive to measure the skin blood flow in anesthetized animal models, movements from breathing can mask the signal of flow. As a consequence, the mean flow is overestimated because a variable amount of a DC component due to the breathing movements is added to the signal of flow. Objective: To evaluate a method for estimation of the background of the signal of flow, rejecting artefacts from breathing. Methods: A method originally developed for accurate DNA sequencing was evaluated. The level of the background signal is estimated in small temporal sliding-windows. The method was applied to evaluate a mouse model of hind limb Ischemia. Skin blood flow from hind limbs of anesthetized C57BL/6 mice (n=13) were measured. The mean fluxes (Fi and Fc from ischemic and control hind limbs) were computed from

the registers of flow and from the corresponding estimated background signals (Fib and Fcb from ischemic and control hind limbs). Results: The mean values of the percentages (a measure of ischemia) MI=(Fi/Fc).100 and MIb=(Fib/Fcb).100 were computed to be 30±4% and 23±3% respectively (mean ± SE) . Evidences of statistical differences between both considered groups were obtained ($p<0.05$, paired student-t). The mean error [(MI-MIb)/MIb].100 obtained was 51±17% (mean±SE). Conclusion: The Recovery of a signal of flow corrupted by artefacts from breathing is feasible, allowing more accurate measurements of the signal of flow.

9531-166, Session PSun

Examination of the variation of the optical diffusion properties in nanophosphor materials for use in biomedical imaging and instrumentation

Panagiotis F. Liaparinos, Ioannis S. Kandarakis, Technological Educational Institute of Athens (Greece)

Micro powder phosphors are commonly used in several applications in biomedical imaging and instrumentation. The structural and optical properties of phosphor materials affect the optical signal transferred out and play a critical role in the quality of the final signal or image. In recent years, with the development of science and technology in the field of materials, several methods have been developed and successfully used for the preparation of nanosized phosphors. It is of interest to investigate whether nanophosphors could replace existing micro phosphors for next generation high-performance displays and imaging devices. The purpose of the present study was to investigate the variation of the optical parameters (e.g. light extinction coefficient, probability of light absorption, light anisotropy factor) in the sub-micron and nanoscale under the variability of light wavelength (400-700 nm) and refractive index (e.g., two limiting values were used 1.4, 2.0). For the case of low refractive index (1.4), by increasing the grain diameter: (a) the light extinction increases, (b) the light absorption probability decreases and (c) the anisotropy factor increases in the whole range or gran sizes (2-1000 nm). However, for the high value of the refractive index (2.0), the light extinction coefficient was found to increase up to a maximum for grain diameter: (a) 200 nm (at 400 nm light wavelength) and (b) 600 nm (at 700 nm light wavelength). Finally, at 400 nm grain diameter, the probability of light absorption was found to decrease down to a minimum while the anisotropy factor was found to increase up to maximum for all light wavelengths considered.

This research has been co-funded by the European Union (European Social Fund) and Greek national resources under the framework of the "Archimedes III: Funding of Research Groups in TEI of Athens" project of the "Education & Lifelong Learning" Operational Programme.

9531-16, Session Pln4

Polarized light in optical biopsy: enabling technologies towards tissue characterization and imaging *(Invited Paper)*

Igor Meglinski, Alexander Doronin, Callum Macdonald, Michael Eccles, Univ of Otago (New Zealand)

We exploit the directional awareness of circularly polarized light. The results of modeling and its cross-validation with the phantom studies, as well as the results of measurements for cancerous and non-cancerous tissue samples are presented. A unified Monte Carlo based computational model for imitation of propagation of coherent linearly and/or circularly polarized light in turbid tissue like scattering media has been developed and extensively used to understand and predict the state of polarization.

9531-43, Session 7

Fabry-Perot microstructured polymer optical fibre sensors for opto-acoustic endoscopy *(Invited Paper)*

Christian F. B. Broadway, Daniel C. Gallego, Univ. Carlos III de Madrid (Spain); Getinet T. Woyessa, Technical Univ. of Denmark (Denmark); Andreas Pospori, Aston Univ. (United Kingdom); Guillermo Carpintero del Barrio, Univ. Carlos III de Madrid (Spain); Ole Bang, DTU Fotonik (Denmark); Kate Sugden, Aston Univ. (United Kingdom); Horacio R. Lamela Rivera, Univ. Carlos III de Madrid (Spain)

Opto-Acoustic Endoscopy (OAE) requires sensor technology with a high sensitivity and small physical dimensions in order to facilitate integration into an endoscope. Optical sensors, such as interferometric fibre sensors, provide significant benefits over conventional piezoelectric sensors such as electromagnetic immunity, higher potential resolution and a large detection bandwidth. With the advent of polymer optical fibres (POF), intrinsic fibre sensors can leverage the improved Young's Modulus compared to silica optical fibre, resulting in more than 12 times improved sensitivity in a given sensor ["High-sensitivity ultrasound interferometric single-mode polymer optical fiber sensors for biomedical applications", Gallego and Lamela, 2009]. Furthermore, POF is well adapted to biomedical use e.g. a non-metallic nature (MRI compatibility) allowing all the benefits of optical fibre with none of the significant risks posed by silica. PMMA microstructured POF has a minimum measured acoustic sensitivity of 9mrad/kPa at ultrasonic frequencies ["Interferometric microstructured polymer optical fiber ultrasound sensor for optoacoustic endoscopic imaging in biomedical applications", Gallego et al, OFS submission 2015], outperforming single mode silica, single mode and graded index polymer fibre.

Fabry-Perot interferometric sensors made from two FBGs are theoretically and experimentally investigated. We model the physical interaction of ultrasonic waves with POF fibres, both with and without inherent structures such as FBGs and F-P cavities. We present an ultrasonic characterisation in the MHz regime to validate the model. Applying the predictive model allows the optimisation of polymer and structure properties for an optimal interferometric OAE sensor configuration.

9531-44, Session 7

The intracellular trafficking pathway of Antibody-conjugated Pulchellin in HIV-infected cells by confocal microscopy

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Immunotoxins (ITs), as reported so far, have targeted mostly hematologic tumors. The available literature on studies targeting solid tumors has shown that though difficult, as tumors can be controlled successfully using these reagents in combination with chemotherapy. ITs are chimeric molecules in which cell-binding ligands are coupled to toxins and can specifically eliminate undesired cells including cancer cells and HIV-infected cells. The cell-binding carriers of anti-HIV ITs have been directed against different regions of the HIV envelope glycoprotein (gp120 and gp41) and surface antigens (e.g CD4, CD25).

Pulchellin is a combination of lectin B subunit and toxic A subunit held together via a disulfide bond. Pulchellin A chain (PAC) is a potent toxin to inhibit the protein synthesis in cancer cells and also HIV-infected cells. PAC, as ribosome-inactivating proteins (RIPs), can be conjugated to monoclonal antibodies to specifically target the HIV-infected cells. To analyze the protein synthesis inhibition by Pulchellin, the intracellular localization of the immunoconjugate should be compared to Pulchellin. In

this case, we can use confocal fluorescence microscopy, to determine cell targeting, endocytosis, intracellular trafficking, and recycling of Pulchellin bioconjugates.

Herein, the use of Antibody-conjugated Pulchellin to eliminate cancer cells and HIV-infected cells is discussed. we will investigate the inhibition of protein synthesis and also induction of cell death specifically in T Cell Tumor lines that are infected with either infectious (H9/HTLV-IIIB) or defective (8E5) HIV. Moreover, possible quantitative methods for fluorescent labeling of the immunoconjugate during confocal microscopy are investigated.

9531-45, Session 7

In vitro evaluation of ionizing radiation effects in bone tissue by FTIR spectroscopy and dynamic mechanical analysis (DMA)

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Ionizing radiation is frequently used in Medicine, such as radiodiagnostic exams, radiotherapy, and sterilization of halograft. It breaks polypeptidic chains, causing the release of free radicals by radiolysis of water, and may change the material mechanical properties. In the specific case of bone tissue, studies report that ionizing radiation induces changes in collagen molecules and reduces the density of intermolecular crosslinks. The aim of this study was to verify the changes promoted by different doses of ionizing radiation in bone tissue using FTIR and dynamic mechanical analysis. Samples of bovine bone were irradiated using Cobalt-60 with five different doses: 0.01kGy, 0.1kGy, 1kGy, 15kGy and 75kGy. To study the effects of ionizing irradiation on the chemical structure of the bone, the sub-bands of amide I, the crystallinity index and relation of organic and inorganic materials, were analysed. The mechanical changes were evaluated using the elastic modulus and the damping value. To verify whether the chemical changes and the mechanical characteristics of the bone were correlated, the relation between the analysis made with spectroscopic data and the mechanical analysis data was studied. It was possible to evaluate the effects of different doses of ionizing radiation in bone tissue. With ATR-FTIR spectroscopy, it was possible to observe changes in the organic components and in the hydroxyapatite crystals organization. Changes were also observed in the elastic modulus and in the damping value. High correlation with statistical significance was observed among (amide III+collagen)/v1,v3 vPO4 3- and the delta tangent, and among 1/FHWM and the elastic modulus.

9531-46, Session 7

Combined Infrared-Fluorescence sensing platform for advanced breath analysis

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Highly integrated sensing platforms have to provide the required sensitivity and molecular selectivity, especially those aiming at medical/clinical applications. Of particular interest are devices that have the potential to perform online non-invasive diagnosis and monitoring tasks in routine clinical environments facilitated by using advanced optical waveguide technology (e.g., substrate-integrated hollow waveguides (iHWG) and optical fibers) as key components [1,2].

The combination of complementary sensing techniques - e.g., as shown herein, FTIR-iHWG and luminescence sensors – allows gathering qualitative and quantitative information on diagnostically relevant breath compounds including volatile organic constituents (VOCs) for analyzing IR-active (e.g., isotope ratio of 12CO2 vs. 13CO2), and IR-inactive species (e.g., O2) in a simultaneous fashion [3,4]. The advantage of this combination resides in the fact that any deviations observed due to the presence of the IR-inactive gaseous species, as observed for analyzing CO2 in the presence of oxygen, can be determined and readily integrated into the calibration model.

The authors thank Science Without Borders (SWB Proc. 407170/2013-8 and 151486/2014-0) for financial support.

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9531-47, Session 7

Assessment of mango fruit ripening using fluorescence spectroscopy

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We are presenting non-invasive assessment of mango ripening using fluorescence spectroscopy. Fluorescence spectra have been acquired from peel of Dusehri mango, a popular cultivar of Pakistan, using a blue LED at 460 nm as an excitation source. It has been observed that instead of chlorophyll fluorescence peak intensity at 680/740 nm, carotenoids fluorescence peak intensity at 540 nm could be used for fruit ripening/maturity assessment. Similar observations have been found in fluorescence spectra of Dusehri pulp using the same experimental setup. It has been further demonstrated that for Langra mango, where peel remains green even at fully ripped stage, chlorophyll based maturity assessment gives false prediction. However, the carotenoid fluorescence signatures are more reliable spectroscopic markers and gives an accurate prediction about the ripening of Langra.

9531-49, Session 7

Optoacoustic processing algorithms for intravascular imaging using polymer optical interferometric ultrasonic sensors

Pablo González, Ehsan Dadrasnia, Omar E. de Varona, Horacio R. Lamela Rivera, Univ. Carlos III de Madrid (Spain)

OptoAcoustic Imaging (OAI) is a new biomedical imaging technology based on the use of laser-generated ultrasound. It offers visualization of optical contrast in tissues, within several millimetres to centimetres, with resolutions that are typical of ultrasound imaging. In many clinical applications, high frequency ultrasounds can only be used in combination with intravascular techniques due to the high acoustic attenuation in organic tissue at tens of megahertz. OAI has the goal of calculating the distribution of the optical absorption coefficient in tissues and requires computer-based reconstruction algorithms. The exact time-domain reconstruction formula produces images with excellent resolution but poor contrast. A wavelet transform implementation using a wavelet family resembling the theoretical N-shaped OA signal can be used to sharpen object boundaries while simultaneously preserving high contrast of the reconstructed objects.

In this work, we present the ultrasonic probe of an optoacoustic intravascular endoscope based on an interferometric polymer optical

ultrasonic sensor, being an important aspect the catheter design, particularly the optimization of the optical and acoustic parameters. To facilitate this process, we develop a model to simulate the ultrasonic pressure field, generated by optical absorption in a physical phantom using an intrinsic ultrasonic optic sensor. We also compare the results provided by these different approximations of OAI with real OA signals collected from the physical phantom design using the polymer ultrasonic optic sensor. Our analysis demonstrates that the image of back-projected wavelet-transformed and simultaneously integrated OA pressure signals possesses the highest contrast and adequate resolution for this OA Intravascular Imaging.

9531-50, Session 8

Frequency components in time-resolved optical signals measured on the surface of the head for assessment of cerebral autoregulation *(Invited Paper)*

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Optical signals measured on the human head reveal fluctuations originating from various physiological processes. We are able to observe pulse waves, respiration-related components and low frequencies connected with the vasomotion. It was shown that time-resolved near-infrared spectroscopy allows for separation of changes in hemoglobins concentration in intracerebral tissue from changes related to extracerebral compartments (like a skin, bones of the skull). Application of the time-resolved technique allows for measurement of this physiological signals fluctuations into the brain cortex tissue and could be potentially useful tool for assessment of brain autoregulation. Frequency analysis of the optical signals and will be applied in the control group of healthy volunteers and in the patients with cerebral blood flow disorders caused by traumatic brain injuries. The instrument is based on semiconductor lasers, photomultipliers and time-correlated single photon counting electronics. The results of frequency analysis of the time-resolved signals showed that in most of the subjects all the statistical moments of the measured distributions of times of flight of photons (number of photons, mean time of flight and variance of the distribution) the components from the pulse and breathing can be observed. This observation suggests that the analysis of these frequency components in the optical signals can be used to study tissue saturation and in combination with the beat-to-beat pressure measurement the methodology may be used for investigation of brain autoregulation.

9531-51, Session 8

Infrared irradiation of skin for the development of non-invasive health monitoring technologies

Gregory E. Triplett, Hisham Abdussamad Abbas, Univ. of Missouri-Columbia (United States)

Infrared radiation was employed to study the optical transmission properties of pigskin and the factors that influence transmission at room temperature. The skin samples from the forehead of piglets were irradiated using an infrared-pulsed source by varying the beam properties such as optical power, power density, duty cycle, as well as sample thickness. Because infrared radiation in select instances can penetrate through thick-fleshy skin more easily than visible radiation, temperature fluctuations observed within the skin samples stemming from exposure-dependent absorption revealed interesting transmission properties and the limits of optical exposure. Pigskin was selected for this study since its structure most closely resembles that of human skin. Furthermore, the pulsed beam technique compared to continuous operation offers more precise control of heat generation within the skin. Through this effort, the correlated pulsed-beam parameters that influence infrared transmission were identified and varied to minimize the internal absorption losses through the dermis layers. The two most significant parameters that reduce absorption losses were frequency and duty cycle of the pulsed beam. Using the Beer-Lambert Law, we approximate the absorption coefficient from empirical data, while accepting that the absorption coefficient is neither uniform nor linear. Given that the optical source used in this study was single mode, the infrared spectra obtained from irradiated samples also reveal characteristics of the skin structure. Realization of appropriate sample conditions and exposure parameters that reduce light attenuation within the skin and sample degradation could give way to novel non-invasive measuring techniques for health monitoring purposes.

9531-53, Session 8

In-vitro visualization of deep blood vessels using laser speckle imaging and PCA

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Laser speckle imaging (LSI) [1,2] is a non-invasive optical technique in which integrated speckle patterns generated by coherent irradiation are imaged with a CCD camera. The analysis of speckle contrast in integrated speckle patterns enables visualization of superficial blood flow in exposed vasculature. Typically, LSI is used to map and quantify relative changes in blood flow velocity in response to an stimulus. Conventional LSI does not enable visualization of deep subsurface blood vessels, because of optical scattering by stationary structures, such as the skull or epidermis. However Photothermal LSI [3] does it, but requires external excitation (595 nm laser pulse) to increase the local blood temperature and hence increased scatterer motion.

Principal Components Analysis (PCA) is an statistical technique that is capable to decorrelate the information in a set of variables called principal components by transforming the original information into PCA domain and preserving only the most significant principal components. In this work we propose and demonstrate the use of LSI in combination with PCA to improve the visualization of in-vitro deep blood vessels without external excitation.

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9531-54, Session 8

3D Monte carlo radiation transfer modeling of photodynamic therapy

Catherine L. Campbell, Christian T. A. Brown, Kenneth Wood, Univ. of St. Andrews (United Kingdom); Ronan M. Valentine, Ninewells Hospital and Medical School (United Kingdom); Harry Moseley, Univ. of Dundee (United Kingdom)

Photodynamic therapy (PDT) is a treatment available for eradication of non-melanoma skin cancer (NMSC) and dysplasia. It relies on the interaction between light and a photosensitive chemical to kill targeted cells. Model based investigations of this process allow for comparison between different methods and conditions of treatment but relies on accurate assumptions and representation of tissue structure and the dynamics of the treatment. We use a sophisticated three dimensional Monte Carlo Radiation Transfer (MCRT) model to simulate the light

passage through skin tissue during different PDT treatment conditions. The concentration of the photosensitive chemical Protoporphyin IX (PpIX) will change with both time and depth and therefore affect the optical properties of the skin tissue.

As an example for the use of this technique, we show that under similar conditions (initial PpIX concentration and light dose delivered) it is possible to treat superficial skin lesions to a depth of 2 mm using daylight as the light source for drug activation. This compares to an effective treatment depth of about 3 mm using conventional treatment methods.

This work shows the importance of the combination of clinical data and mathematical modeling to successfully investigate skin structures, optical properties and different treatment methods.

9531-55, Session Pln5

Global endoscopy: opportunities and challenges in endoscopic imaging and early cancer detection

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Over the past 6 decades, the field of endoscopy has revolutionized medicine by allowing clinicians to access and treat regions of the body (bladder, oropharynx, airway, GI tract) in a more efficient and minimally invasive fashion. In gastroenterology, advances in optical imaging and engineering have led to the development of advanced endoscopes that allow enhanced visualization of mucosal and vascular patterns and subcellular changes that can delineate and differentiate neoplasia in real time. These, however, have led to increased, arguably, unsustainable costs in the field of endoscopy.

This lecture will discuss these advances in the context of global gastrointestinal endoscopic demand and elaborate on some potential cost-effective approaches for cancer detection in lower resource areas worldwide.

9531-56, Session 9

Multiplexing intracellular redox potential and pH measurements in 3D breast cancer tumour models using SERS nanosensors *(Invited Paper)*

Lauren E. Jamieson, The Univ. of Edinburgh (United Kingdom); Aleksandra Jaworska, Jagiellonian Univ. in Krakow (Poland); Pierre O. Bagnaninchi, Jing Jiang, Kate Fisher, The Univ. of Edinburgh (United Kingdom); David J. Harrison, Univ. of St. Andrews (United Kingdom); Colin J. Campbell, The Univ. of Edinburgh (United Kingdom)

Cellular redox potential is incredibly important for the control and regulation of a vast number of processes occurring in cells. When the fine redox potential balance within cells is disturbed it can have catastrophic consequences. Of particular interest to my research is the redox gradient that develops in cancer tumours where the internal regions are further from vascular blood supply and therefore become starved of oxygen and hypoxic. This makes treatment of these areas a lot more challenging as chemotherapy approaches rely on the presence of oxygen and with a poor vascular blood supply, drugs delivered through the blood stream will have poor access to these regions

To aid the development of drugs and therapies to overcome this problem a system that gives a quantitative map of redox potential changes through a tumour would be a vital tool. Our technique uses redox sensitive molecules attached to gold nanoshells (NS), which are delivered to cells and give signals using Surface Enhanced Raman Scattering (SERS). As redox potential is pH dependent a system that multiplexes pH and redox measurement by SERS was developed. Imaging techniques including Photothermal Optical Coherence Tomography (OCT), Transmission Electron Microscopy (TEM), Helium Ion Microscopy and Confocal Fluorescence Microscopy have been used to investigate intracellular NS distribution and overall 3D NS distribution in the multicellular tumour spheroid (MTS) models. New techniques combining Raman and Light Sheet Microscopy (LSM) are being investigated to allow decreased acquisition time and increased z penetration in the thick MTS models.

9531-57, Session 9

Biochemical changes in cutaneous squamous cell carcinoma submitted to PDT using ATR-FTIR spectroscopy

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Photodynamic therapy (PDT) is a promising alternative for pre-cancerous lesions and non-melanoma skin cancer treatment as cutaneous squamous cell carcinoma (SCC), which is the second most common skin cancer due to its aggressive and potentially metastatic characteristics. In this work we used Attenuated Total Reflectance - Fourier Transform Infrared Spectroscopy (ATR-FTIR) and Histopathological analysis to evaluate the biochemical and morphological changes after 10 and 20 days of PDT in SCC. The pharmaceutical cream used is based in lanolin and the active principle being either aminolevulinic acid - ALA (20%) and other ingredients kept confidential (patent pending PIN°0705591-9). SCC was induced in Swiss mice by chemical carcinogenesis model and submitted to PDT using a prototype consisting of a cluster with 30 LEDs (Light Emission Diode) emitting in 630 nm, output power of 180mW and fluence of 5 mW/cm2. Histopathological analysis showed an evident reduction of the epithelium thickness, ulceration and extensive epithelial necrosis after 10 days of PDT. At 20 days post-PDT the papillary pattern was not visible and the connective tissue exhibited intense reparative process. ATR-FTIR spectra shown alterations in the absorption peaks related to CH2/CH3 stretching bands (lipids), amide I and II (proteins), RNA and carbohydrate bands in the 3100-2800 cm-1 region of the spectrum. Based on histopathological evaluation we can conclude that there is a significant reduction in neoplastic lesions after 20 days of PDT treatment whereas ATR-FTIR spectroscopy evidenced biochemical changes in the absorption bands associated to the components of the skin samples.

9531-58, Session 9

Optical diagnosis of HCV infection in human blood sera using Raman spectroscopy

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Hepatitis C virus (HCV) infection in human blood is a serious issue in Pakistan. Currently existing methods are either costly (PCR based) or suffering from the issues of false positive/negative (ELISA). We are interested to develop Raman spectral markers of HCV infection which could be used for the optical diagnostic purposes.

9531-59, Session 9

Applications of Raman spectroscopy in life science

Airton A. Martin, Claudio A. Téllez, Said M. Ali, Lazaro P. Medeiros, Renata A. Canevari, Univ. do Vale do Paraíba (Brazil)

Raman spectroscopy has been applied to the analysis of biological samples for the last 12 years providing detection of changes occurring at the molecular level during the pathological transformation of the tissue. The potential use of this technology in cancer diagnosis has shown encouraging results in the in vivo, real-time and minimally invasive diagnosis. Confocal Raman technics has also been successfully applied in the analysis of skin aging process providing new insights in this field. The topic of this presentation will be on the latest biomedical applications of Raman spectroscopy in our laboratory. We will show that Raman spectroscopy (RS) has been used for biochemical and molecular characterization of thyroid tissue by micro-Raman spectroscopy and gene expression. This study aimed to improve the discrimination between different thyroid pathologies by Raman and gene expression analysis. A total of 35 thyroid tissues samples including normal tissue (n=10), goiter (n=10), papillary (n=10) and follicular carcinomas (n=5) were analyzed. The gene expression analysis was performed by qRT-PCR technique for TG, TPO, PDGFB, SERPINA1, LGALS3 and TFF3 genes and statistically analyzed by Mann-Whitney test. The confocal Raman spectroscopy allowed a maximum discrimination of 91.1% between normal and tumor tissues, 84.8% between benign and malignant pathologies and 84.6% among carcinomas analyzed. It will be also presented the in vivo Raman data from human skin focusing in the aging effect in the collagen structure.

9531-60, Session 9

Optical pathology diagnosis of brain cancer by native fluorescence and Stokes shift spectroscopy

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Optical pathology is based optical spectroscopy method including native fluorescence, Stokes shift, multiphoton fluorescence images and special frequency analysis.

In this report, Optical pathology was applied to diagnose human brain cancer in vitro. The study concentrated on identification of brain cancer from normal tissues by native fluorescence and Stokes shift spectra (SSS).

Thirty five Brain specimens including three type brain tissues (normal, primary tumor and metastatic brain carcinoma) from five grades were studied.

In order to observe fluorescence spectral changes of fluorophores, a wide excitation wavelength from UV, to visible (260 to 530 nm) for emission spectra and a different Stokes shift spectra with intervals Δ? from 10 to 120 nm were measured.

The fluorescence spectra and SSS from multiple key native molecular markers, Tryptophan, Collagen, NADH, Alanine, Ceroid and Lipofuscin, were observed in normal and disease brain tissues. Two diagnostic criteria were established based on the ratios of the peak intensities and peak position in both spectra of fluorescence and Stokes shift. It is observed that the ratio of the spectral peak intensities of tryptophan (337 nm) to NADH (443 nm) was increased in glioma and malignant meningeal tissues in comparison with in normal tissues, and the ratio of the SSS peak (Δ? =40nm) intensities of 292 nm to 366 nm was incremented in the process with the increasing grades of glioma malignant.

An MCR-ALS algorithm method used in spectral data analyses, and a predictions accuracy rate is 0.80 in comparison with histopathology and immunohistochemistry reports (gold standard).

9531-61, Session 9

Localization of subsurface fluorescent lesions using surface spectral measurements

Kolbein Kolste, Stephen C. Kanick, Thayer School of Engineering at Dartmouth (United States); Pablo A. Valdes, Brigham and Women's Hospital (United States) and Boston Children's Hospital (United States); Brian C. Wilson, Univ. of Toronto (Canada); David W. Roberts M.D., Dartmouth Hitchcock Medical Ctr. (United States); Frédéric Leblond, Ecole Polytechnique de Montréal (Canada); Keith D. Paulsen, Thayer School of Engineering at Dartmouth (United States)

Extent of tumor resection increases the survival rate of cancer patients. 5-aminolevolunic acid induced protoporphyrin IX fluorescence is currently undergoing trials for use in brain tumor resection as a way of maximizing completeness of resection while minimizing the damage done to normal tissue. Most research has focused on the use of blue light excitation of PpIX to visualize the tumor, but this work aims to demonstrate the feasability of supplementing the blue light with red light excitation to detecting fluorescence at depth. Previous studies have shown that using diffuse imaging, the depth of fluorescent biomarkers can be estimated by quantifying the deformation of the detected fluorescence spectra due to the wavelength-dependent light attenuation by overlying tissue. This is done by using a normalized fluorescence emission ratio and knowledge of the optical properties of the diffusing medium. This study uses liquid phantoms to determine the maximum depths at which this technique can be used for <1mm depth estimation for concentrations ranging from 0.01 μg/mL to 10 μg/mL and optical properties found in vivo (μa=0.01-0.1 cm-1 and μs'=10-20 cm-1). Topographic maps were created to localize the source of the fluorescence and indicate the depth. Two separate inclusions at different depths were able to be differentiated. Additionally, a tumor model in rats was used to demonstrate in vivo that the topographic maps correlate with the tumor boundaries. Finally, a first look at depth estimation using the dual wavelength fluorescence ratiometric technique in human is presented.

9531-62, Session 9

Human dental enamel sterilization by gamma radiation aimed in situ use

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The in situ model involves the use of intraoral device that creates defined conditions in the oral cavity in humans without causing interference in their natural dentition. Thus, taking in account the biosafety of volunteers, biological samples should be submitted to disinfection or sterilization before use in situ. Gamma irradiation appears to be the most suitable method for sterilization of biological samples once that leaves no residue unlike immersion techniques that use antimicrobial materials. At the present study, approved by Ethics Committee, 72 donated HDE samples were irradiated through a product overlapping source at Co60 Multipurpose Irradiator at IPEN aiming complete sterilization (2 cycles of 12,5KGy/h). After irradiation, HDE samples were submitted to percentual microhardness loss to analyze the mineral content; electronic microscopy scanning morphological analysis; biological essays to bacteria, mold and yeast to certify the HDE sterilization; and by Fourier Transform Infrared spectroscopy (FTIR) to analyse molecular changes at hidroxyapatite crystalline pattern, previously and after gama irradiation.

Results shows that gama radiation applied to HDE sterilization purposes seems to be safe and causes minimal changes at the mineral content and morphological aspects of enamel samples. These findings contribute to a bias free results concerning to the sterilization process at enamel samples preparation and also minimizes ethical concerns when assures the volunteers health, which may also increase their adhesion to the in situ model.