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14. ABSTRACT

This report covers a Center grant comprised of nine project areas focused on developing advanced photonic systems for acute care and trauma medicine. All nine projects share common objectives in developing either diagnostic, therapeutic, and/or analytical photonic tools that are of interest to military and civilian medicine and that help solve complex problems of emerging national need. The projects outlined in the report include:

- Development of High Speed Functional OCT
- Airway Injury, Part I: An In Vitro Model of Toxic Gas Exposure
- Airway Injury, Part II: High Resolution Fiberoptic F-OCT Imaging in Acute Inhalation Injury.
- Airway Injury, Part III: High Resolution F-OCT and MPM/SHG Imaging of the Oral-Nasal Cavity
- Diffuse Optical Spectroscopy in Critical Patient Medicine
- Optical Therapeutics in Cartilage for the Treatment of Traumatic Injuries and Degenerative Disease

15. SUBJECT TERMS
Optical Imaging, Optical Coherence Tomography (OCT), Non-linear Optical Imaging (NLOM), Multiphoton Microscopy (MPM), Airway Imaging, Joint Imaging, Mustard Gas, In-Vitro Models, Diffuse Optical Spectroscopy, Hemorrhage Monitor, Critical Care Monitor, Near-infrared Imaging, Cartilage Reshaping, Bone and Cartilage Ablation, Traumatic Brain Injury, Internet-based Robotic

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Final Performance Report

PRINCIPAL INVESTIGATOR: Michael W. Berns, Ph.D.

INSTITUTION: University of California, Irvine

GRANT TITLE: Advanced Optical Technologies for Defense Trauma and Critical Care

GRANT NUMBER: FA9550-04-1-0101

AWARD PERIOD: (15 February 2004 – 30 September 2008)

Project #1: Development of High Speed Functional OCT

PRINCIPAL INVESTIGATORS: Zhongping Chen, Ph.D., and J. Stuart Nelson, M.D., Ph.D.

OBJECTIVE: The objective of this research is to develop a compact, high-speed, phase-resolved functional optical coherence tomography (F-OCT) instrument that can simultaneously obtain in situ tissue structure, blood flow, birefringence, and spectroscopic images with high spatial resolution. The system will be used in imaging injury and wounds in skin, airway, and joint cartilage.

APPROACH: There are three limitations in our current prototype OCT, PS-OCT, and ODT systems that are being addressed. 1) Multiple functional imaging is performed by separate systems. An integrated F-OCT system that can simultaneously provide information on tissue structure, birefringence, blood flow, and spectroscopic properties will greatly enhance its utility. 2) We are developing a high-speed, fiber optic based high-resolution F-OCT system to increase speed of data acquisition, and to integrate Doppler, polarization, and spectroscopic image modalities. 3) We are developing a portable broadband light source that can achieve high speed and high spatial resolution simultaneously.

ACCOMPLISHMENTS: We have developed a compact, high-speed, phase resolved functional OCT system. The system is based on a wide bandwidth Fourier domain mode-locked (FDML) wavelength swept laser using two gain media. The wavelength swept laser is capable of FWHM scanning range of more than 135 nm at 45 kHz sweeping rate. The Fourier domain OCT based on this swept laser achieves both high resolution (4.7 μm) and high speed (90 kHz) simultaneously. In addition, we developed two scanning probes that enable high-speed 3-D imaging. One is based on dual-axis MEMS mirror that provides linear 2-D scanning and the other is based on a rotational micro-motor that provides helical 2-D scanning. We have demonstrated in vivo applications of these probes for endoscopic 3-D OCT imaging. Finally, we have

developed a high resolution second harmonic OCT system that provides additional molecular contrast for OCT images. More than 40 manuscripts have been published under the full or partial support of this grant. The following are highlights of the accomplishments:

(i) **High-resolution second harmonic OCT of collagen in rat-tail tendon (Applied Physics Letters 86, 133901, 2005).** A high-resolution second harmonic OCT (SH-OCT) system was demonstrated using a spectrum broadened femtosecond Ti:sapphire laser. An axial resolution of 4.2 μm at the second harmonic wave center wavelength of 400 nm has been achieved. Because the SH-OCT system uses the second harmonic generation signals that strongly depend on the orientation, polarization and local symmetry properties of chiral molecules, this technique provides unique contrast enhancement to conventional OCT.

(ii) **3-D endoscopic OCT by use of a two-axis MEMS scanning mirror (Applied Physics Letters 88, 163901-3, 2006).** We present a three-dimensional (3-D) endoscopic optical coherence tomography (OCT) system based on a dual axis scanning microelectromechanical system (MEMS) mirror. The diameter of MEMS mirror is 1.2 mm and both axes are capable of scanning greater than 20° with linearity. The endoscopic MEMS probe was integrated with an OCT system and volume images were obtained at a rate of 3 frames/s by means of 2-axis lateral scanning combined with an axial scan.

(iii) **Improved lateral resolution in Fourier domain OCT by 2-D numerical diffraction method (Optics Express 15, 7634-41, 2007).** We present a non-iterative, two-dimensional numerical method to alleviate the compromise between the lateral resolution and wide depth measurement range in OCT. A 2-D scalar diffraction model was developed to simulate the wave propagation process from out-of-focus scatterers within the short coherence gate of the OCT system. High-resolution details can be recovered from outside the depth-of-field region with minimum loss of lateral resolution. Experiments were performed to demonstrate the effectiveness of the proposed method.

(iv) **High-speed and wide bandwidth Fourier domain mode-locked wavelength swept laser with multiple SOAs (Optics Express 16, 2547-54, 2008).** We reported on the development of a high-speed, wide bandwidth Fourier domain mode-locked (FDML) wavelength swept laser of around 1300 nm using two gain media for high-resolution and high-speed Fourier domain OCT. The wavelength swept laser is capable of FWHM scanning range of more than 135 nm at 45.6 kHz sweeping rate. The measured axial resolution of the forward scan is 6.6 μm in air and 4.7 μm in tissue. The peak power is 11.4 mW for both the forward and backward scans. The measured system sensitivity is achieved up to 100.7 dB. We also demonstrated OCT imaging using the FDML wavelength swept laser with two semiconductor optical amplifiers.

(v) Real-time swept source OCT imaging of the human airway using a MEMS endoscope and digital signal processor, (Journal of Biomedical Letters, 030506, 2008). A fast-scan-rate swept laser for optical coherence tomography (OCT) is suitable to record and analyze a 3-D image volume, however, the system speed is limited by data streaming, processing, and storage. In this case, post-processing is a common technique. Endoscopic clinical applications prefer onsite diagnosis, which requires a real-time technique. Parallel digital signal processors were applied to stream and process data directly from a data digitizer. A real-time system with 20 kHz axial line speed, which is limited only by our swept laser scan rate, was implemented. To couple with the system speed, an endoscope based on an improved 3-D microelectromechanical motor (diameter 1.5 mm, length 9.4 mm) was developed. In vivo 3-D imaging of human airway was demonstrated.

SIGNIFICANCE: F-OCT has great potential for use in the clinical management of combat injuries and wounds in skin, airway and joint cartilage. Raster scanning the probe allows reconstruction of high resolution (2-10 μm) "optical biopsy" of the structure and perfusion of injured tissues. The potential to image rapidly and accurately, and to distinguish viable from nonviable tissue over large areas, would be of enormous benefit to the military burn surgeon. The integrated F-OCT system will enhance the imaging capabilities of OCT for clinical applications.

Student Theses:

1) Jianping Su, Ph.D., August 2008

Thesis Title: Development of endoscopic and second harmonic optical coherence tomography

Summary: This research focuses on endoscopic OCT diagnosis. Because of the high-resolution capabilities of OCT technology, its use for endoscopic diagnostics aims to perform "optical biopsy" without damaging the tissue, detect early stage diseases and monitor the healing process after surgery. The organs and tissues such as blood vessel, vocal cord, gastrointestinal track and airway are investigated. In order to access these organs, four OCT endoscopes were designed to deliver infrared light to the region of interest. First, a flexible OCT endoscope is designed to image neonatal vocal cord. Second, a coil reinforced OCT endoscope was designed to image human airway. Third, an endovascular OCT imaging probe was designed to image blood vessels and the first OCT image of *in vivo* human post-treatment cerebral aneurysm was obtained. Forth, a MEMS motor-based OCT endoscope was designed to image the gastrointestinal track. In addition to endoscope development, a high speed, real time, swept source OCT system was designed using digital signal processor in order to perform real time *in vivo* imaging. This system enables real time two dimensional image slices acquisition at 20 kHz A-line rate. The three dimensional

reconstruction of these slices shows detailed morphological tissue structure that provides better diagnostic information than a two dimensional image.

Finally, a second harmonic spectrometer based OCT was designed to increase OCT image contrast. With the contrast enhancement from second harmonic generation, the structure of highly organized tissue, like collagen, is obtained. This information is complementary to the standard OCT. Spectrometer based system decreases the imaging time 10 to 100 times compared to the previous time domain system. SH-OCT has the potential to provide molecular contrast for in vivo clinical application.

2) Woong Gyu Jung, Ph.D., September 2008

Thesis title: Development of 3-D endoscopic OCT and Fiber Based Multi-Photon Microscopy using Miniaturized Imaging Probes

Summary: OCT is a cross-sectional imaging method based on the detection of backscattered near infrared light from tissue. It is capable of non-invasive, high resolution imaging in real time. OCT has been used for endoscopic imaging using fiber-optic probes that intrinsically offer easy integration with clinical endoscopes. Currently, most of probe-based images are restricted to 2-D. However, it is difficult for clinicians to find the exact location and extent of a diseased site with 2-D information. 3-D OCT images can provide instant visualization of the size and extent of the diseased tissues making the imaging procedures more accurate and faster. The first section of this thesis covers the development and investigation of an advanced 3-D endoscopic OCT system. In particular, the endoscopic probe in this study was designed using 2-axis scanning microelectromechanical system (MEMS) mirror which supports rapid scanning, compact size, high reliability, and flexibility in scanning pattern. Feasibility studies were then performed on various animal and human tissues *in vivo*.

OCT typically images tissue morphology with contrast derived from tissue scattering, whereas multiphoton microscopy (MPM) provides information of tissue functionality. MPM is based on nonlinear multi-photon excitation of fluorophores generated at the focal point of the microscope objective. It permits high resolution, non-invasive images of cellular and extracellular matrix at depths of several hundred microns within tissues. Studies of probe-based MPM are still under evaluation because of the engineering challenges such as miniaturization of scanning probe. The second section of thesis focuses on the design and implementation of a miniaturized MPM probe. In order to resolve key challenges of such a probe, a MEMS scanning mirror and a double-clad photonic crystal fiber (DCPCF) were utilized. The use of a MEMS mirror and a DCPCF provides many advantages, such as size reduction, rapid and precise scanning, efficient delivery of short pulses, and high collection efficiency of fluorescent signals. The completed probe was integrated into an MPM system and used to image fluorescent beads, paper and biological specimens. Engineering solutions developed in these

studies provide knowledge and enable accumulation of experience that will aid in successful transitioning of optical imaging platforms from the laboratory setting to the clinical environment.

Project #2: Airway Injury, Part I: An In Vitro Model of Toxic Gas Exposure

PRINCIPAL INVESTIGATORS: Steven George, M.D., Ph.D.

OBJECTIVES: The objective of this research is to characterize the response of an in vitro tissue model of the lung epithelium to chlorine inhalation injury, and evaluate the potential use of this model for the study of other chemical and biological warfare agents.

APPROACH: Our initial plan included harvesting lung tissue from male pathogen-free Sprague-Dawley rats for in vitro culture. However, we found that commercially-available normal human bronchial epithelial cells can be cultured to more effectively simulate a human airway epithelium. This tissue model of the bronchial epithelium was then exposed to different doses of chlorine (concentrations x exposure time). To characterize the response of the tissue model to different doses of chlorine, we utilized Western blot and ELISA to quantify the production of proteins such as MUC5AC, a major component of mucus, and important growth factors such as TGF-β. We also use RT-PCR to assess mRNA expression of structural and inflammatory proteins. In addition, the barrier property of the epithelium was assessed by measuring the transepithelial electrical resistance (TER).

ACCOMPLISHMENTS: During the first year, we worked on setting up our equipment for the experiments, which included a new incubator to perform our gas exposure, and modifying our experimental design to eliminate the use of animals.

During the second year, we developed and optimized our model of the airway epithelium using cultured normal human bronchial epithelial cells. These advances resulted in an improved viability of the epithelial cell monolayer, which also displayed the phenotypic features of the in situ epithelium, including cilia, cuboidal morphology, tight junctions, and mucus production.

During the 3rd and 4th year, we performed a number of chlorine exposures on our tissue culture model. The epithelial cell monolayers were exposed to 20 ppm of chlorine for 3-6 hours. The chlorine exposure occurred in a dedicated incubator housed within a fume hood. Using a flow meter, the chlorine gas was delivered at 40mL/min and a chlorine detector was used to verify the concentration of gas. We sacrificed the tissues at 0, 24, and 48 hours following the exposure. We monitored

transepithelial electrical resistance (TER) and examined the production of several key proteins using Western blot and ELISA; however, no clear trends were observed as a result of the chlorine exposure. This may be due to the inter-subject/donor variability of normal human bronchial epithelial cells.

Because nitric oxide (NO) activity has been implicated in chlorine-induced lung injury, we also monitored NO production and the acitivity of nitric oxide synthase (NOS: the enzyme that catalyzes the formation of NO). Using a NO analyzer and the Griess colorimetric assay, we measured the NO produced by the cell monolayers exposed to chlorine. We found no difference in NO production between chlorine-exposed cells and control cells. Similarly, Western blot analysis of the inducible form of NOS did not show a difference between chlorine-exposed cells and controls.

STUDENT THESES:

Nikita K. Malavia, Ph.D., September 2008

Theseis Title: Airway epithelium mediated structural cell remodeling

Summary: Ms. Malavia worked on the development and optimization of our model of the airway epithelium using cultured normal human bronchial epithelial cells. She used this model to study the effects of pro-inflammatory cytokine exposure on the airway epithelium. She found that prolonged IL-13 exposure followed by withdrawal creates an epithelial phenotype, which continuously secretes TGF-ß2 at levels that increase collagen secretion and stimulates fibrosis. Hence, IL-13 may contribute to subepithelial fibrosis in asthma by stimulating biologically significant TGF-β2 secretion from the airway epithelium.

Project #3: Airway Injury, Part II: Optical Characterization, Diagnostics, and Treatment Evaluation. High Resolution Fiberoptic F-OCT Imaging in Acute Inhalation Injury.

PRINCIPLE INVESTIGATORS: Matt Brenner, M.D., and Zhongping Chen, Ph.D.

OBJECTIVES: The objective of this research was to develop flexible fiberoptic optical coherence tomography methods of high resolution endobronchial imaging for assessment of inhalation airway injury. Improved methods for airway injury assessment are essential for advancing management of inhalation injury from burns, smoke inhalation, toxic gas exposures, and biological inhalation agent exposures. Factors affecting airway injury determination include structural changes, edema, epithelial sloughing, as well as changes in airway epithelial blood flow. The focus of this research section was to develop, translate and

validate methods for flexible fiberoptic functional optical coherence tomography of acute inhalation airway injury. The studies are closely coordinated with optical and cellular investigations of the pathophysiologic changes occurring concurrently with the functional OCT changes.

APPROACH: The specific aims of this work were to translate F-OCT technology for diagnosis and treatment of airway injury (toxic and burn/thermal) in rabbit smoke inhalation injury and half mustard injury models, and in large animal (sheep) chlorine inhalation injury. During these investigations, the correlation between the extent and depth of inhalation damage determined from structural OCT was compared to histological and clinical findings. We have also continued investigations of traumatic epithelial lining induced changes.

ACCOMPLISHMENTS: During the course of these studies, we have developed a <1 mm diameter prototype translational longitudinal flexible fiberoptic probe for bronchoscopic endobronchial high resolution OCT/ODT applications, and OCT and histologic evaluations of normal trachea and bronchi. The proposed series of smoke inhalation and mustard airway injury animal studies were completed. Animals were treated with controlled smoke exposures, and real time, 3D-OCT images were obtained documenting the epithelial and subepithelial changes (as compared to controls) for 6 hours post treatment. Dramatic mucosal and submucosal swelling was demonstrated with continuous OCT monitoring. OCT images revealed that tracheal swelling occurs within minutes of exposure, much faster than previous literature has reported. This research was presented at the International ACCP meetings last year and was a finalist for the "Outstanding Research Award". These studies demonstrated significant differences in response to injury in upper versus lower trachea, and a close correlation between smoke dose exposure and injury in the lower trachea/bronchi in contrast to the more proximal trachea. Manuscripts describing these studies have been published in the Journal of Biomedical Optics. In the ½ mustard injury model, dramatic changes were seen within minutes of mustard exposure, with mucosal edema, hemorrhage, and sloughing of epithelium evident by OCT and confirmed by histology. We began investigating cellular changes, mediator and cell communication events using brush specimens obtained under direct visualization to correlate the OCT optical events with underlying cellular and molecular changes occurring after injury in both smoke and mustard injury animals.

High resolution detection of inhalation airway injury using OCT in the chlorine inhalation injury sheep model at the USAISR was started with the airway research group from BLI and the research groups of Drs. Lee Cancio and Andriy Batchinsky at USAISR. We worked to develop methods to study OCT of inhalation injury burn patients at USAISR. In collaboration with Drs. Cancio and Batchinsky, we also began to investigate diffuse optical spectroscopy (DOS) measurements on the inhalation chlorine lung

injury animals. DOS measurements were made concurrently with OCT measurements to provide information on systemic perfusion and tissue oxygenation in a combined inhalation injury hemorrhage model.

SIGNIFICANCE: Inhalation/burn airway injury and toxic gas inhalation injury are major health hazards for military, civilian, and firefighting personnel. Thermal injury, vesicants, and other toxic agents cause airway edema, hyperemia, mucosal injury, and may lead to airway obstruction. Recognition of early airway changes is essential to improving management, triage, and treatment of airway injury. OCT and ODT may offer the possibility of radically changing the approach to inhalation injury treatment by providing a high resolution optical technology for early diagnosis in such potentially lethal exposures. Early sensitive, quantitative, minimally invasive methods for improving determination of airway injury also have the potential for providing methods for determining the effectiveness of proposed treatment methods.

Project #4: Airway Injury, Part III: Optical Characterization, Diagnostics, and Treatment Evaluation. High Resolution F-OCT and MPM/SHG Imaging of the Oral-Nasal Cavity

PRINCIPAL INVESTIGATORS: Petra Wilder-Smith, D.D.S., Ph.D., and Zhongping Chen, Ph.D.

OBJECTIVES: The upper airway is uniquely accessible for multimodality high resolution imaging to provide information on the extent of exposure and risks to lower airway injury. Using real-time fiberoptic functional F-OCT and MPLSM of the upper airway (naso-, oropharynx), our group has developed a capability for early diagnosis of oral/nasal inhalation injuries. Our recent work demonstrates that multimodality high resolution upper airway imaging can provide early, simple, accurate assessment of inhalation injury and treatment needs and allow easier monitoring of response to therapy, leading to an overall improvement in patient care and recovery. Based on that work, this proposal will quantify precisely the relationship between upper airway inhalation injury, diagnosis and treatment and the entire airway.

APPROACH: Using the hamster and rabbit airway HMG models the time-based structural and perfusion effects of upper and lower airway injury were investigated. Specifically, the relationship between time-based development of effects and the severity of these effects in the upper vs. the lower airway at defined exposure concentrations and durations were defined. F-OCT data from excised airway specimens and in-vivo imaging were augmented with additional high resolution information on structural, collagen, ECM, and vascular changes from MPLSM/SHG imaging. Imaging data was compared to conventional histopathology and immunohistochemistry.

ACCOMPLISHMENTS: First, hamster and rabbit models for a vesicant exposure were developed and non-invasive f-OCT and MPLSM imaging protocols were established. From these studies, a close correlation was identified between (1) HMG damage to the upper airway (naso-pharynx) and the lower airway and (2) optical mapping of airway damage and conventional histopathology, immunohistochemistry. After initially using doses and exposure durations cited in the literature as being near the minimal threshold for vesicant effects on airway mucosa (5-1 mg/ml, for 1-5 minutes), it became apparent that lower doses and exposure durations also needed to be investigated. At intervals of 5 minutes to 24 hours after exposure to HMG, at exposure concentrations as low as 0.2 mg/ml for as little as 15 seconds, non-invasive MPLSM and OCT imaging demonstrated tissue responses including increasingly severe blistering, membrane cloudiness, swelling of muscle layer and severe disruption of muscle fiber layer. Effects in the upper airway were predictive of effects in the lower airway with a kappa of 0.93. OCT and MPLSM of individual tissue layers demonstrated dose and time dependent differences in tissue response, with effects in surface layers showing a closer relationship to exposure duration, and subsurface effects demonstrating a greater dose-dependency.

SIGNIFICANCE: Optical techniques are exceedingly sensitive to HMG-induced damage in airway tissue. F-OCT and MPLSM in the oral-nasal cavity can characterize very early inhalation injury in the entire airway at exposure levels below those identified using conventional, ex vivo techniques. Our non-invasive imaging approach translates into a rapid, non-invasive tool for clinical use that is extremely sensitive to inhalation injury and that can be readily used as often as needed to evaluate injury development over time as well as treatment response.

Project #5: Diffuse Optical Spectroscopy in Critical Patient Medicine

PRINCIPAL INVESTIGATORS: Matthew Brenner, M.D., Bruce J. Tromberg, Ph.D., Albert Cerussi, Ph.D., Jangwoen Lee, Ph.D., Jae Gwan Kim, Ph.D., Rick Convertino, M.D., Andiy Batchninsky Ph.D., Thomas J. Walters, Ph.D.

OBJECTIVE: The main goals of this project were to provide optical monitors of trauma encountered in critical care and battlefield medicine. Specifically, our plan was to provide real time, non-invasive, in vivo quantitative measurements of (a) hemoglobin derivatives and tissue perfusion during hemorrhage and other injuries, and (b) hemodynamic changes and physiologic parameters during cyanide toxicity and reversal, and to develop and test a potential novel effective intramuscular injection treatment for acute cyanide toxicity that may radically alter treatment and prevention of cyanide poisoning.

APPROACH: Diffuse optical spectroscopy (DOS) is used to quantify tissue chromophore concentrations (oxy-, deoxy-, and Meth-hemoglobin, water, lipid, cytochrome oxidation and other NIR absorbing solutes) to determine tissue perfusion and metabolic states under various insults. Non-invasive in vivo measurements of tissue perfusion from DOS are compared with conventional clinical evaluation standards to determine the utility of non-invasive DOS diagnostics in critical care patient medicine.

ACCOMPLISHMENTS: Investigations were conducted using DOS to monitor tissue perfusion states during hypovolemic hemorrhage and various protocols of resuscitation (saline, autologous whole blood, and poly-heme blood substitutes). The findings from these investigations were published, and additional findings are currently in the process of publication. We have also published results of diffuse optical spectroscopy (DOS) monitoring of hydroxocobalamin treatment of cyanide toxicity and reported interference by hydroxocobalamin with standard optical cooximetry.

We used DOS to non-invasively monitor the effects of cyanide exposure on tissue oxygenation and DOS measurements were validated using on-site cooximetry. DOS detection of CN toxicity treatment with therapeutically induced methhemoglobinemia and hydroxocobalamin (OHCOb) was investigated. We have demonstrated that the effects of cyanide toxicity can be monitored noninvasively, treatment can be followed, and the effectiveness of treatment can be assessed very accurately using DOS. We demonstrated that hydroxocobalamin is much more rapid and effective than met hemoglobin formation in reversing a range of aspects of cyanide toxicity. Findings from these works have been published in abstract form, and are currently in the process of publication. Most importantly, we have begun a series of investigations into a novel compound, cobinamide, that has the potential to reverse cyanide toxicity. Cobinamide is highly soluble, stable, and can be concentrated to the degree that it may be usable as an IM or inhaled antidote for cyanide toxicity. We have completed a series of initial investigations into dosing, delivery, effectiveness, and kinetics of cyanide reversibility using cobinamide monitored by DOS. We have shown the effectiveness of both I.V. and aerosolized delivery of the drug in our rabbit model. These methodologies have the potential to radically change the treatment options for cyanide exposure victims.

We initiated continuing studies on the ability of DOS to measure tissue oxygenation changes following the use of vasoactive agents. We chose four common vasoactive agents, norepinephrine, phenylephrine, dobutamine, and nitroprusside and are currently investigating the effects of these vasoactive agents on tissue oxygenation and correlation between DOS measurements and conventional hemodynamic measurements. We also expanded our research beyond animal studies. Human clinical trials of trauma and critically ill patients are currently under way at the

surgical and medical intensive care unit at University of California, Irvine Medical Center.

In collaboration with Dr. Vic Convertino at USAISR, we performed a series of studies on volunteers undergoing lower body negative pressure (LNBP) simulation of hemorrhagic shock. A very close correlation was demonstrated between noninvasive optical DOS measurements of indicators of reduced tissue perfusion versus stroke volume measurements and other physiologic findings. These promising results suggest that DOS may provide a noninvasive method for assessment of hemorrhage in human beings. The results of these studies have been accepted for publication in the Journal of Biomedical Optics.

Continuous wave near-infrared spectroscopy studies of tourniquet injury performed with Dr. Tom Walters at USAISR have demonstrated the ability to detect reversible versus irreversible ischemic changes in small animals by CWIS compared to pathologic findings. A manuscript describing findings from this study is being finalized at this time.

SIGNIFICANCE: DOS instrumentation can be tailored for specific clinical applications, can provide real-time diagnostic capabilities equal or superior to those of current standard practice, and can aid in critical care and battlefield patient assessment of adequacy of resuscitation, recovery and survival. The use of DOS may dramatically improve our capabilities for detecting injuries, as well as our ability to determine the effectiveness of novel treatment regimens.

Project #6: Optical Therapeutics in Cartilage for the Treatment of Traumatic Injuries and Degenerative Disease

PRINCIPAL INVESTIGATORS: Brian J.F. Wong, M.D., Ph.D., J. Stuart Nelson, M.D., Ph.D., Dmitry Protsenko, Ph.D.

OBJECTIVE: The objectives of this study were broadly focused on developing laser cartilage reshaping technologies with the aim of clinical translation. The study focused on understanding basic mechanisms for reshaping and identifying the relationships between clinically relevant shape change and laser dosimetry. The clinical target applications include the correction of traumatized and damaged nasal, auricular and tracheal structures.

APPROACH: The approach in this study has been a series of "bench to bedside" developmental tasks focused on clinical translation of laser cartilage reshaping technology. Research has focused on three clinical areas of reshaping (nasal, ear, tracheal). Basic investigations were performed primarily to aid in the optimization of the reshaping process.

ACCOMPLISHMENTS: During the past four years significant accomplishments have been made in four major areas: 1) Mechanisms and mathematical modeling; 2) Animal model; 3) Clinical/translational studies; and 4) Spinoff technologies.

Modeling efforts have focused on developing an opto-thermo-mechanical model to describe the shape change process and it is a first of its kind. This was implemented using real human data derived from CT images of the nasal vault and coupled to a thermal injury model. The model estimates shape change and thermal injury in septum after laser irradiation. It assists in identification of optimal irradiation pattern covering internal stress concentrations and selection of laser power and irradiation time for clinically relevant shape change with minimal thermal injury. The model was verified using thermal imaging and precision mechanical measurements.

Fundamental research has focused on the characterization of the transitional temperature required for permanent shape change and elastic properties of septal, auricular and tracheal cartilage during and after laser irradiation. This was balanced with experimental studies to determine the effect of dosimetry on tissue viability. Process optimization focused on the development of cooling methods to control thermal injury during reshaping.

In vivo investigations using animal models were performed as a first step toward clinical studies. Major work focused on the development and characterization of septal cartilage, trachea, and auricular reshaping in the rabbit model. The latter project featured the use of cryogen spray cooling and is likely the first spin-off technology that will be commercialized for reshaping.

Clinical-translation work has focused on the development of two applications: 1) Auricular cartilage reshaping; and 2) Septal reshaping. The septal reshaping work has focused on determining dosimetry for accomplishing shape change in cartilage and has included pilot investigations in cadaver tissues to aid in device development. Additional work has focused on electron microscopy of cartilage as well, and examining viability as a function of dosimetry. Pilot clinical evaluation was also performed. Progress toward developing auricular reshaping has focused on performing measurements of composite human auricular cartilage tissues, and developing a laser-cryogen system to perform this reshaping of the human ear in vivo.

<u>Two spinoff technologies</u> were derived from reshaping technology research: 1) electromechanical reshaping (EMR) and 2) photothermal cartilage regeneration. In EMR, in situ redox chemistry is initiated in cartilage tissue leading to changes in tissue internal stress and resulting in sustained shape change. Photothermal regeneration is a process that we hypothesize would be feasible for a non-ablative optical "microfracture" that uses

pulsed IR heating to create microscopic regions of tissue damage in hyaline cartilage that then may undergo regeneration. In the final year of support, a third spinoff of laser reshaping was developed with a focus on "warping stabilization" in costal (rib) cartilage tissues.

Student Theses:

Yong-Seok Chae, Ph.D., June 2005.

Thesis Title: The Thermal Behavior of Cartilage

Summary:

This doctoral thesis performed the first comprehensive thermal analysis of cartilage tissue using methods primarily used in materials science research. The major conclusions include: 1) The enthalpic relaxation in cartilage occurs over a broad temperature range; 2) The process is highly dependent upon tissue water content; 3) Two enthalpic events occur when cartilage is heated thru the range of temperatures in which reshaping occurs; and 4) T
he relationship between temperature, shape change, and tissue mechanical properties in cartilage is exceedingly complex.

Project #7: Advanced Optical Technologies for Orthopedic Applications

PRINCIPAL INVESTIGATORS: George M. Peavy, D.V.M., Brian Andrews M.D., Tuqiang Xie Ph.D., Zhongping Chen Ph.D.

OBJECTIVES:

This project had two thrusts in orthopedic applications: 1) Tissue ablation and 2) Minimally invasive joint imaging.

The objectives of ablation were to define an appropriate wavelength, energy levels and delivery mode for the development of laser systems for orthopedic surgical applications.

The objectives in tissue imaging were to assess the ability to image normal and pathologic joint tissue (articular cartilage and synovium) by Optical Coherence Tomography (OCT) and Multiphoton Microscopy (MPM) imaging technologies for minimally invasive diagnostic applications.

APPROACH:

Tissue Ablation: An FEL tunable to wavelengths in the infrared spectrum was used to ablate bone and articular cartilage using a consistent pulse structure and variable pulse energies to determine the ablation efficiency and effects on cortical bone and articular cartilage. From initial studies, the wavelengths

of 2.79, 2.94, 6.1 and 6.45 μm were selected for further evaluation of the ablation characteristics and to test hypotheses regarding laser ablation of bone and cartilage.

Minimally Invasive Joint Imaging: Ex vivo specimens of normal and pathologic articular cartilage and synovial tissue were imaged by table top OCT, PS-OCT and MPM systems and these images were compared to histology preparations of the same tissue specimens. Rigid and flexible probes are being developed to allow arthroscopic imaging of patients using OCT and MPM technologies.

ACCOMPLISHMENTS:

Tissue Ablation: Specimens of bone and articular cartilage were irradiated at the Vanderbilt University FEL Center in comparative ablation studies involving the identified wavelengths of interest 2.79, 2.94, 6.1 and 6.45 μm. Findings from this work demonstrated most efficient bone cutting with the least amount of thermal injury (less than 10 μm) when using the 6.1 μm wavelength delivered in pulses of less than 10 μsec duration, and were the subject of several publications.

Imaging of normal and pathologic articular cartilage and synovial tissue specimens by OCT, PS-OCT and MPM imaging technologies have been completed and published. OCT and PS-OCT imaging of normal and pathologic specimens has demonstrated the ability to image characteristics of cartilage indicative of early disease processes and late stage organizational changes that have not been reported previously for OCT and PS-OCT imaging.

PS-OCT images compared to polarized microscopy evaluation of the collagen fibril orientation of normal articlar cartilage specimens has demonstrated normal variations in fibril orientation in normal cartilage specimens that dramatically influence the PS-OCT imaging and may confound the interpretation of PS-OCT imaging of joint cartilage if the normal fibril orientation of a joint is not understood and mapped as a clinical reference.

MPM imaging studies have demonstrated the ability to visualize and differentiate structural and cellular components of the synovium in fresh, unprocessed and unstained tissue. These are the first studies to be reported for MPM imaging of joint capsule and synovial tissue. We have begun MPM imaging of pathologic synovial tissue obtained from rabbit models of immune mediated and septic inflammatory joint disease, and are comparing these to histology sections of the same specimens. It appears that MPM will allow high resolution imaging of the cellular and matrix components of fresh joint tissue without the need for harvesting and processing tissue specimens.

Imaging probes for arthroscopic application have been designed and are currently under construction.

Publication of accomplishments have been included in each of the annual progress reports for this project, and there are no additional publications to report at this time.

Project #8: Optical and Molecular Approaches to the Study of Chemical Agents (Sulfur Mustard) on Cell Cytoskeletons

PRINCIPAL INVESTIGATOR: Michael W. Berns Ph.D.

OBJECTIVE: The main goal of this project has been to gain a basic understanding of the effects of chemical defense agents (i.e.: sulfur mustard/half-mustard) on cell structure and function, and to develop new microscope hardware (RoboLase) to perform these studies.

APPROACH: In order to study the cytoskeletal effects of sulfur mustard, modern optical and molecular/cellular approaches were developed and employed. A secondary approach was to develop internet-based communication and a robotic laser microscope, "RoboLase," to facilitate these studies.

ACCOMPLISHMENTS: Our studies have demonstrated that sulfur half-mustard (CEES) has discrete molecular effects on cells with short exposures. In brief, we have seen discrete cytoskeleton changes in response to 5 minute exposures to CEES. Cells expressing a tubulin-GFP fusion protein 1) have exhibited dramatic disorganization in their microtubule and filament structure; 2) the data suggest that these changes are regulated in a cell cycle fashion; 3) at a low CEES dose the cell cytoskeleton recovers; and 4) we have demonstrated that cells removed directly from the airway following exposure to CEES for five minutes, exhibit disruption in the internal cytoskeleton similar to what was observed in the model cell culture system. These observations and conclusions are part of a multi-investigator collaborative study with another grant module and will be contained in a joint publication that obtained it's major support from this grant (see Project #3: Airway Injury, Part II: *Optical Characterization, Diagnostics, and Treatment Evaluation of High Resolution Fiberoptic F-OCT Imaging in Acute Inhalation Injury*).

In addition the RoboLase microscope has been developed for internet-based collaboration. This system was built with funds provided as a part of supplemental funding to the present grant and resulted in a major publication (Botvinick and Berns, Microsc. and Res. tech. 68:65-74, 2005). The system allows laser microscopes at either UCI/BLI or UCSD to communicate with each other directly via the internet, or either system to communicate with anyone around the world via an internet-based "logmein" service. The system has been tested using fast moving biological

cells (sperm) controlled from as far away as Australia. In the course of perfecting RoboLase, software and hardware interfaces have been developed that permit the tracking of fast moving objects followed by automatic robotic laser interception and trapping of the object. In conclusion, the results of the 4 years of research under this module of the grant has been 10 peer reviewed articles, 6 review chapters, and 8 published abstracts/meeting presentations.

Student Theses:

Jaclyn Nascimento, Ph.D., January 2007

Thesis Title: Analysis of Sperm Motility and Physiology using Optical Tweezers

Summary: The purpose of this dissertation was to first develop an objective and quantitative method to analyze sperm motility based on sperm swimming force. A custom RoboLase system was perfected to track, trap, and fluorescently image sperm, measuring sperm swimming speed, swimming force and mitochondrial membrane potential in real-time. This system was then used to study sperm. Specifically, the effects of trap duration and laser trapping power on sperm motility were determined, the relationships between sperm swimming force and swimming speed for various mammalian species were defined, both the effects of cryopreservation and sperm competition in primate species on motility were studied, and finally the relationship between sperm motility (swimming force and swimming speed) and energy production was analyzed. In relation to the AFOSR grant, this thesis served to develop and validate many aspects of the RoboLase microscope system. Particularly, the hardware, software, and the ability to track and trap fast moving objects served to validate the major elements of the system.

Project #9: Non-Invasive Optical Imaging of Brain Trauma and Neuroprotection

Principal Investigators: Bruce J. Tromberg, Ph.D., Enrico Gratton, Ph.D., William W. Mantulin, Ph.D., Ron Frostig, Ph.D.

OBJECTIVES: Direct, quantitative assessment of brain trauma arising from battlefield injury is a major challenge in combat casualty care. Beyond characterization of acute injury, long term treatment and follow up of brain trauma is equally challenging. The broad objectives of our program are: 1) develop strategies that could improve triage of head injuries under battlefield conditions, 2) monitor the appearance and progression of trauma-induced brain pathologies, and 3) assess the efficacy of neuroprotective strategies.

We proposed a collaborative study between Beckman Laser Institute and WRAIR that would:

1) Establish quantitative, non-invasive optical imaging endpoints that correlate with gold-standard measures of injury.
2) Develop a portable non-invasive optical system for animal studies at WRAIR.
3) Test a portable system for human use in trauma, stroke, and epilepsy patients.

APPROACH: Our approach has relied on a duel strategy. The first part has concerned development of hardware systems such as the modulated imaging (MI) and diffuse optical spectroscopy (DOS) along with the associated software for instrument control, data acquisition and data analysis/visualization. The second component of our strategy has focused on development of robust animal models for producing, detecting and monitoring ischemia related to cortical spreading depression (CSD), middle cerebral artery occlusion (MCAo), transient ischemia (TIA), and elevated intracranial pressure (ICP). The characterization of these models is accomplished by MI and DOS. The translation of these concepts to the non-invasive observation of human brain trauma is to be accomplished with DOS.

ACCOMPLISHMENTS: Our strategy combined technical development of optical instrumentation (hardware and software), followed by system testing in animal models of trauma, and culminating with assessment of neurotrauma in humans. Modulated Imaging (MI) using spatially modulated (i.e. structured) broadband light was used to generate spatial and temporal maps of tissue absorption, scattering and fluorescence properties. MI can be used in an intraoperative setting to image through intact or thinned skull preparations (mouse and rat models, respectively) and during craniotomies for human subjects. Using a prototype MI device, we have completed rat model studies that separate and quantify the absorption and reduced scattering coefficients of brain in the 650-1000 nm wavelength range. During the past 18 months we have assembled a dedicated brain imaging team (neurosurgeons, neurobiologists, engineers, physicists, chemists) and developed an MI system to characterize brain function in standard injury models: cortical spreading depression (CSD), transient ischemia (TIA) and middle cerebral artery occlusion (MCAo). We have published the results of these studies.

Student Theses:

David Cuccia, Ph.D., 2006

Thesis Title: Modulated Imaging: A Spatial Frequency Domain Imaging Method for Wide-field Spectroscopy and Tomography of Turbid Media in Biomedical Engineering.

Summary: The thesis laid out the fundamental theoretical and experimental parameters for accomplishing optical imaging in tissue with spatial modulation; referred to as Modulated Imaging

(MI). This work was validated in a series of brain imaging experiments in a live animal model, which demonstrated that MI was quantitatively able to track traumatic brain injury events such as cortical spreading depression and stroke. MI has now moved on to further developments in neurosurgery applications.