Attachment to form DOE F 241.1

K. DESCRIPTION/ABSTRACT

This Final Report summarizes the significant progress made by the researchers, students and staff of the *Center for Laser Imaging and Cancer Diagnostics* (CLICD) from January 1998 through May 2002. During this period, the Center supported several projects. Most projects were proposed initially, some were added subsequently as their relevance and importance to the DOE mission became evident. DOE support has been leveraged to obtain continuing funding for some projects. Leveraged funds come from various sources, including NIH, Army, NSF and the Air Force.

The goal of the Center was to develop laser-based instruments for use in the detection and diagnosis of major diseases, with an emphasis on detection and diagnosis of various cancers. Each of the supported projects is a collaborative effort between physicists and laser scientists and the City College of New York and noted physicians, surgeons, pathologists, and biologists located at medical centers in the Metropolitan area. The participating institutions were: City College of New York Institute for Ultrafast Lasers and Spectroscopy, Hackensack University Medical Center, Lawrence Livermore National Laboratory, Memorial Sloan Kettering Cancer Center, and New York Eye and Ear Institute.

Each of the projects funded by the Center is grouped into one of four research categories: a) Disease Detection, b) Non-Disease Applications, c) New Diagnostic Tools, and, d) Education, Training, Outreach and Dissemination.

The progress achieved by the multidisciplinary teams was reported in 51 publications and 32 presentations at major national conferences. Also, one U.S. patent was obtained and six U.S. patent applications have been filed for innovations resulting from the projects sponsored by the Center.

The final report is comprised of a summary of the work performed and results obtained for the various Center projects and, as attachments, the Progress Reports submitted previously for 1998, 1999, 2000, and 2002, as well as a comprehensive listing of publications presentations and patents arising from work funded by the Center. Although funding from the Department of Energy has ended, work continues on many of the projects, through funding leveraged from other funding sources. Continuing projects are noted as such in the text of this report. Continuing funding has been requested from DOE and is still required to complete those projects for which we have not been able to leverage funding.

DISCLAIMER

Center for Laser Imaging and Cancer Diagnostics

A U.S. Department of Energy Center for Excellence in Laser Medicine

Final Report

October 31, 2002

DOE Patent Clearance Granted
Jay Ahn for Mark Druskovich
14 May 2003

GRANT NO. DE-FG02-98ER62560

PRINCIPAL INVESTIGATOR

Robert R. Alfano, Ph.D.
City College of New York
Ph: 212-650-5531 Email: alfano@sci.ccny.cuny.edu

CO-PROJECT INVESTIGATOR

Jason A. Koutcher, M.D., Ph.D.
Memorial Sloan-Kettering Cancer Center
Ph: 212-639-8834 Email: koutcher_jason/mskccmail.mskcc.org

Contents

Introduction

This Final Report summarizes the significant progress made by the researchers, students and staff of the *Center for Laser Imaging and Cancer Diagnostics* (CLICD) from January 1998 through May 2002. During this period, the Center supported several projects. Most projects were proposed initially, some were added subsequently as their relevance and importance to the DOE mission became evident. DOE support has been leveraged to obtain continuing funding for some projects. Leveraged funds come from various sources, including NIH, Army, NSF and the Air Force grants.

The goal of the Center was to develop laser-based instruments for use in the detection and diagnosis of major diseases, with an emphasis on detection and diagnosis of various cancers. Each of the supported projects is a collaborative effort between physicists and laser scientists and the City College of New York and noted physicians, surgeons, pathologists, and biologists located at medical centers in the Metropolitan area. The participating institutions were: City College of New York Institute for Ultrafast Lasers and Spectroscopy, Hackensack University Medical Center, Lawrence Livermore National Laboratory, Memorial Sloan Kettering Cancer Center, and New York Eye and Ear Institute.

Each of the projects funded by the Center is grouped into one of four research categories: a) Disease Detection, b) Non-Disease Applications, c) New Diagnostic Tools, and, d) Education, Training, Outreach and Dissemination.

The progress achieved by the multidisciplinary teams was reported in 52 publications and 32 presentations at major national conferences. One U.S. patent was obtained and six U.S. patent applications have been filed for innovations resulting from the projects sponsored by the Center.

The final report is comprised of a summary of the work performed and results obtained for the various Center projects and, as attachments, the Progress Reports submitted previously for 1998, 1999, 2000, and 2002, as well as a comprehensive listing of publications presentations and patents arising from work funded by the Center. Although funding from the Department of Energy has ended, work continues on many of the projects, through funding leveraged from other funding sources. Continuing projects are noted as such in the text of this report. Continuing funding has been requested from DOE and is still required to complete those projects for which we have not been able to leverage funding.

A. Disease Detection

Breast Cancer Detection
Staff: S.K. Gayen, Ph.D., R.R. Alfano, Ph.D., W. Cai, Ph.D., M. Lax, Ph.D., M. Alrubaiee (graduate student), Y. Budansky (Laser Engineer) CCNY; J.A. Koutcher, MD, PhD, Memorial Sloan Kettering Cancer Center; H.E. Savage, New York Eye and Ear Infirmary
Period: 1998-2002

The goal of this project was to develop a safe and affordable breast cancer screening modality that uses noninvasive and non-ionizing optical radiation to image and diagnose cancerous lesions of human breast. Major advances towards achieving the goal were made through the successful

completion of a majority of the tasks set in the original proposal. We have already leveraged funding (Army IDEA grant *Breast Cancer Screening Using Photonic Technology*, 8/98-9/01) and hope to continue work with a renewal proposal, *Two-Dimensional and Three-Dimensional Optical Mammography*, which was already submitted. Accomplishments to date include:

- Demonstration that the time-sliced imaging approach can distinguish between normal and cancerous excised human breast tissue.
- Development of spectroscopic imaging approach and demonstration of diagnostic potential.
- Validation of optical and spectroscopic imaging measurements.
- Derivation of analytic solutions of scalar and vector Boltzmann Radiative Transport Equations.
- Development of 3-D inverse image reconstruction algorithms for transmission and back-propagation geometries;
- Reconstruction of 3-D inverse image from time-resolved 2-D data;
- Development of a time-resolved Fourier diffuse optical tomography approach;
- Development of a faster and more accurate photon transport forward model.
- Development of a stochastic view of photon migration in turbid media.
- Demonstration of correlation between optical measurements and the NMR approach.
- Extension of the time-resolved and spectroscopic imaging approach.
- Assembly of optical mammography bed for in *vivo* study on volunteers (has not been used to date – need to obtain additional support in order to begin *in-vivo* studies).

Correlation of NMR and Optical Imaging Results (Part of Breast Cancer Detection Program)
Staff: J. A. Koutcher, M.D., Ph.D., S. Xu; Memorial Sloan-Kettering Cancer Center (MSKCC)
Period: 2000

The goals of this study were to 1) supply normal and cancerous breast tissue for imaging by both laser spectroscopic techniques and magnetic resonance imaging for comparison, and 2) determine if laser spectroscopic imaging correlates with the results of 31P NMR spectroscopy. To date we have studies 5 samples using NMR spectroscopy and optical imaging.

Spectroscopic and optical time-sliced imaging measurements were carried out at CCNY on the samples provided by MSKCC. A tissue sample holder was constructed to enable measurement using NMR and optical techniques. Optical measurements correlated well with NMR results.

Early Prostate Tumor Detection Using Spectral Polarization Imaging
Staff: W.B. Wang, R.R. Alfano, Ph.D., Ph.D., J.H. Ali (graduate student), CCNY; J.H. Vitenson, J.M. Lombardo, N. Sherman, L. Rammazotto, Hackensack University Medical Center (HUMC).
Period: 1998-2002

The goal of this project was to develop a spectral polarization imaging modality for early prostate tumor detection. We built and used a breadboard instrument for preliminary work conducted at HUMC where the unit is located. We began development of a spectral polarization

imaging diagnostic device. We succeeded in obtaining continuing funding for this project from the Army Medical Research and Materiel Command (*Prostate Cancer Detection using Near IR Spectral Polarization Imaging*, 7/01-6/04, $536,956). To date on this project we have:

- Measured the fluorescence spectra of prostate cancerous and normal tissues, and imaged prostate samples using the fluorescence difference imaging method.
- Measured the absorption spectra for human prostate and rectum tissues.
- Designed and built a breadboard spectral polarization imaging unit, and tested human and animal tissue samples and veins.
- Transferred the prototype spectral polarization imaging unit to HUMC from CCNY, and re-aligned for imaging measurements.
- Obtained more than ten human in *vitro* prostate tissue samples from both HUMC and the National Disease Research Interchange (NDRI) and provided the optical imaging studies
- Investigated prostate samples using the near infrared spectral polarization imaging unit.
- The spectral polarization imaging measurements were performed on two rectum-membrane-prostate tissue samples.
- Measured more than ten model human rectum-membrane-prostate-object samples with different optical imaging methods including scattering imaging, tissue emission imaging and contrast agent emission imaging. The guest objects hidden inside the prostate tissues beneath the rectum-membrane structure at depths of 2-5 mm were identified.
- Built an IR spectral polarization imaging setup using a PbS and InGaAs CCD camera.
- This project also resulted in new, non-medical, research – detection of corrosion underneath paint layers on metal surfaces – for which funding has been obtained from NSF and AFOSR.

Detection of Eye Diseases Using Raman Spectroscopy
Staff: A. Katz, Ph.D., R.R. Alfano, Ph.D., G. Minko (undergraduate student), CCNY; R.B. Rosen, M.D., E. Kruger, M.D., New York Eye and Ear Infirmary
Period: 2000-2002

This project had two goals. The first goal was to investigate the feasibility of using Raman spectroscopy to detect the presence of molecules, such as glutamate, which are indicators of diseases in the eye. The second goal was to investigate the use of light scattering to identify common eye bacteria based on differences in the elastic scattering properties of the bacteria.

Detection of Glutamate by Raman Spectroscopy
Raman measurements were performed on *ex vivo* porcine eyes and potential problems in extracting meaningful Raman signal have been identified. The major problems were: (1) presence of fluorescence wing background and (2) large Raman signal from proteins in the lens and cornea. An optical system has been designed which significantly reduces the background Raman signal from the lens and cornea that can interfere with detection of the glutamate signal. Glutamate was injected into *ex vivo* porcine eyes to simulate disease conditions and the Raman spectrum was collected using the new optical system. The results from this project indicate that clinical application of this technology would require significant improvement in detection sensitivity.

Raman and Elastic Scattering from Bacteria

In this project, the Raman and elastic scattering properties of some common bacteria were studied in order to determine if spectroscopic signatures could uniquely identify different types of bacteria. Preliminary Raman measurements were performed on bacterial solutions at NYEEI. The solutions exhibited large backscatter of the exciting laser light, no Raman lines could be identified in the acquired spectrum. Spectral and angular dependence of elastic scattering from suspensions of different types of bacteria in saline was investigated and compared to theoretical calculations in order to determine if measurements of scattering properties can accurately distinguish different types of bacteria. Preliminary measurements were performed and the data analyzed. We obtained some funding to continue this project, in part, under a new NASA Center grant, *University Research Center for Optical Sensing and Imaging.*

B. Non-Disease Applications

Laser Tissue Welding

Evaluation of Laser Tissue Weld Strength

Staff: T.K. Gayen, M.D., A. Katz, Ph.D., R.R. Alfano, Ph.D., CCNY; H.E. Savage, Ph.D., S.A. McCormick, MD., New York Eye and Ear Infirmary.

Period: 1998-2002

This study had three components: a) Evaluation of weld strength as a function of wavelength, b) Study of tissue molecular changes following LTW, and c) search for gelatin-based solder agents.

a) The goal of this work was to develop a suitable near infrared (NIR) tunable laser tissue welding technique that uses various wavelengths. We achieved full thickness tissue welding with *ex-vivo* aorta and skin samples. The welding of aorta was so perfect that it was difficult to distinguish the welded sites from the non-welded sites on hematoxylin and eosin staining of the samples under optical microscope. No collateral damage of the aorta samples was observed. Histopathological study of the welded samples were performed by Dr. Savage and Dr. McCormick at NYEEI. Scanning electron microscopic study of the welded samples supported the optical microscopy results. A computer controlled tissue welding station was developed to make the tissue welding process operator independent. Also an optical fiber delivery system for the Cr^{4+} laser was developed. Both devices are currently being used for LTW. We were successful in obtaining funds from the NIH (Heart Blood and Lung Program) to continue this project (*Near Infrared Tunable Laser Tissue Welding*, 9/00-8/03, $204,587).

b) The goal was to detect molecular changes, in *situ,* following laser tissue welding, using native fluorescence spectroscopic imaging. Fluorescence spectroscopy was used to non-invasively detect the distribution and quantitative changes in collagen and elastin in the welded tissue. Test results showed a reduction of collagen and elastin emission in the fluorescence images of the welded region. The result revealed that optical spectroscopic imaging offers a new in *situ* noninvasive detection technique for molecular evaluation of laser tissue welding. Over the past year under NIH support in part, we performed fluorescence imaging study of the welded pig aorta and skin samples using the same excitation and emission wavelengths for collagen and elastin respectively. The fluorescence intensity from the inner surface and cross section of the weld region was very uniform across the sample with no indication of the presence of a weld.

c) The task was to develop new gelatin-based solders for laser tissue welding using Cr^{4+}-based lasers. Studies concentrated on identifying the optimum constituents of gelatin-based solders that yield the strongest tissue bonds. The final goal was to improve this laser welding technique for future clinical application. Gelatin was esterificated with a fatty acid (ex. myristic acid). Different weight ratios of gelatin to fatty acid were mixed and treated at different temperatures under gentle stirring in a water bath. An *ex vivo* study of tensile and torque strengths with different types of solders was undertaken. A gelatin-based solder called Power-gel© was developed by our group at City College. The new solder has a tensile strength up to nine times greater and torque strength up to ten times greater than that of a 50% albumin solution, which is the currently used solder.

Hormone Levels Probed by Fluorescence Spectroscopy
Staff: Alvin Katz, Ph.D. R. R. Alfano, Ph.D., CCNY; Frederick Naftolin, M.D., D.Phil., Paolo Rinaudo, M.D., Yale School of Medicine
Period: 2000-2002

The goal was to develop a method to monitor, non-invasively, hormone levels using fluorescence spectroscopy. Fluorescence signatures from estrogen sensitive tissue (i.e. external female genitalia) are correlated with hormone levels in women at different times of the menstrual cycle. The changes in fluorescence are caused by changes in collagen and elastin. Fluorescence measurements are non-invasive and results are available in real-time. The technology may eventually be developed for in-home monitoring of the ovarian cycle. Measurements on patients are underway at Yale New Haven Hospital under the direction of Prof. Frederick Naftolin. Fluorescence data were acquired from volunteer female subjects over a two-month duration. Spectral measurements were performed on the vulva and, as a control, the forearm. Preliminary analysis revealed that the emission intensity from the vulva exhibited large day to day fluctuations during ovulation while the spectral data from the forearm exhibited no such fluctuations. Additional series of measurements are needed to confirm these results and to investigate if probe location on the vulva can influence measurements. Fluorescence data was acquired from patient volunteers at the Department of Reproductive Medicine at Yale. New Haven Hospital and the data compared to hormone levels as determined by blood analysis. This study has been terminated due to intellectual property issues which have yet to be resolved.

Study of Sleep-to-Wake Transition Using Near Infrared Spectroscopy
Staff: G. Zhang, Ph.D. (graduate student who received doctorate for work on this grant), A. Spielman, Ph.D., Y. Budansky, R.R. Alfano, Ph.D., CCNY
Period: 2000-2002

The goal was to use near infrared spectroscopy (NIRS) to study the hemodynamics of brain tissue associated with sleep–to–wake transitions in healthy patients. An initial NIRS experimental setup has been improved to enhance its stability and signal-to-noise ratio. The performance of the continuous wave type NIRS device was compared with a commercialized frequency domain type device using a newborn piglet animal model. The result demonstrated very good correlation with the relatively lower cost instrument, and has been published. The results show that a synchronized, parallel change in oxy-Hb and deoxy-Hb is a discrete event that occurs in the transition from both sleep to wakefulness and wakefulness to sleep. The major source of changes measured in this study is the cerebral cortex at the frontal pole. No support to continue this project has been identified.

Study of Sleep Apnea Using Near Infrared Spectroscopy
Staff: A. Spielman, Ph.D., G. Zhang, Ph.D., R. R. Alfano, Ph.D., Y. Budansky; CCNY
Period: 2000-2002

The goal was to find the relationship between the metabolism rate of the human brain and the extent of apnea status in patient subjects, and use the NIRS method with a pulse oxymeter and EEG device to find the relationship between the metabolism rate of the human brain and extent of apnea status in apnea patients. A modified device could be used in hospitals to monitor this link in patients. While the preliminary results are limited to observations in one patient with obstructive sleep apnea (OSA) Syndrome, data suggest that the dynamic changes in oxygenation produced by OSA are different in the peripheral and central compartments. No support to continue this project has been identified.

C. New Diagnostic Tools

Subsurface Spectral Polarization Difference Imaging as a Diagnostic Tool for Medical Applications
Staff: S.G. Demos, Ph.D., H.B. Radousky, Ph.D., LLNL; R.R. Alfano, Ph.D., CCNY
Period: 1998-2002

The object of this research was to develop a subsurface optical imaging method that could offer enhanced imaging depth (up to 1cm). Our approach uses different illuminating wavelengths in combination with polarization techniques to obtain images of subsurface tissue structures at different depth zones based on their difference in absorption, scattering and depolarization.

Our goal was to demonstrate deep subsurface imaging in tissue systems which has been achieved and most of the important aspects of the technique have been explored. Such technology may be used for imaging on skin to detect and image structures of interest located on the skin or below the skin. It can also be incorporated into endoscopes to reach the interior of the human body. These applications can be used as a screening tool (e.g. cancer detection) significantly enhancing the information obtained using existing endoscopic technology. Finally, it can be used during surgery to assist the doctor to target or avoid certain tissue structures (such as nerves or veins).

During this research effort, a number of experimental imaging systems were built and tested in order to explore the abilities and limitations of our approach. Utilizing the 700-1000 nm spectral region, we were able to demonstrate deep subsurface imaging of an object located up to 1.5 cm below the surface of a host animal tissue. Imaging depth was found to depend on the scattering properties of the host animal tissue – in chicken breast tissue, the maximum imaging depth was found to be approximately 1.5 cm while in bovine muscle tissue it was found to be approximately 0.5 cm. The image resolution also depends on the scattering properties of the host tissue. This is not surprising since this technique utilizes the image information carried by diffusive photons in order to satisfy the requirement for large imaging depth.

Experiments have shown that using a low power diode laser for illumination, the image acquisition time can be of the order of 10 milliseconds, suggesting that this method can be used for real time imaging. Our experiments also demonstrated the ability of the technique to provide information regarding the depth of the object, which in effect allows for 3-dimensional mapping.

Spectral Polarizing Tomographic Dermatoscope
Staff: R.R. Alfano, Ph.D., Alvin Katz, Ph.D., Y. Budansky, J.C. Luo; CCNY
Period: 1999-2002

The goal was to develop a hand-help Spectral Polarizing Dermatoscope (SPD) for enhanced imaging of subsurface skin lesions. This method uses the spectral and depolarization properties of more deeply penetrating light to enhance imaging of lesions. Screening for skin cancer was performed using low power magnification (~8-10x) and index matching oil to reduce scattering at the stratum corneum-air interface. This allows the pigmented structures of the epidermis, dermoepidermal junction and dermis to be visualized, and the characterization of different morphological features indicative of different types of skin cancer. The SPD uses multispectral illumination (red, green, and blue LEDS, and white light) and rotatable polarizers to improve the visualization of subsurface features in lesions. A radio frequency interface will transfer images to a computer for storage and additional processing. Prototype SPD units have been constructed using various cameras (RGB and monochrome). The images from the different SPD units will be evaluated to determine color fidelity, color channel crosstalk, spatial resolution and signal-to-noise levels of the different units.

Spectral Interference Tomography
Staff: A. Gilerson, Ph.D., Y. Yan, CCNY; H. Ishikawa, M.D., New York Eye and Ear Institute
Period: 1998-2000

The original project goal was to create a grating-generated interferometric tomography system without scanning in a reference arm of the interferometer. This goal has been achieved by creating a laboratory setup based on data acquired with a charge coupled device (CCD) camera and diffraction grating in the reference arm. The development of this setup could lead to the creation of an optical imaging technique for ocular imaging capable of yielding high resolution cross-sectional images in ultra-fast scanning with minimum moving parts. The technique can be used to diagnose malicious tissue in the eyes. It can be also used to monitor lesions in the skin including skin melanoma located on the surface and at depths up to 2 mm. We expect that a working prototype can be ready in approximately one year.

During the grant period several interference tomography setups with diffraction gratings were tested to provide optimal resolution, dynamic range, scanning time, and durability. The depth-lateral images of test objects and biological tissue, obtained without moving parts, were digitally reconstructed from several CCD camera images. Advanced image reconstruction algorithm was developed. Analysis of image characteristics acquired with different devices led to the proposal for a fast scanning unit based on a commercially available CCD digital line scan camera. A laboratory CCD prototype camera was setup with an 80db dynamic range and resolution about 15 μm, has been developed.

Nonlinear Optical Histology and Tomography
Staff: F. Liu, R. R. Alfano Y. Guo (graduate student) (CCNY)
Period: 1998-1999

This project aimed to develop microscopic imaging tools for tissues on a cellular level using nonlinear optical signals such as SHG (Second Harmonic Generation) and TPF (Two Photon Fluorescence). The goal was to image changes associated with disease progression at early

stages. As tissues are highly scattering media to image through, it is important to understand the light propagation of these optical signals.

Subsurface structures of animal tissue were imaged using SHG and TPF scanning microscopic techniques to reveal the spatial distribution of molecular symmetry and composition. Changes in molecular symmetry associated with tumor progression were demonstrated in carcinogen agent-treated hamster check pouch tissue models. The generation and propagation of re-emission of nonlinear optical signals in turbid media were investigated for various active scattering media. Scattering reduces the optical sectioning ability of the nonlinear optical signal. The detected signals no longer directly reflect the local optical properties of the focal region. It was found that direct nonlinear optical (NLO) imaging may be limited to 4-5 scattering mean free path lengths beneath the tissue surface. A theoretical model was developed to explain the intensity profile as a function of focal depth and scattering strength.

Tunable Solid State Lasers
Staff: V. Petricevic and R. R. Alfano (CCNY)
Period: 1998-2002

The goal of this project was to develop and optimize tunable solid-state lasers for use in medical diagnostics and tissue welding. The performance of the continuous-wave, all-solid-state, double-clad fiber, laser-pumped Cunyite (Cr^{4+}:Ca_2GeO_4) laser and the Cr: YAG laser were improved by developing better quality laser crystals and employing novel pump laser sources. Continuous-wave and mode-locked operation of cunyite using fiber laser pump source was demonstrated. The continuous-wave Cr^{4+}:Ca_2GeO_4 laser yielded maximum output power of 415 mW at 1420 nm and tuning range of 1335-1492 nm. With a saturable-absorber mirror, 60-ps pulses and 110-mW maximum average output power were generated from a passively mode-locked Cr^{4+}:Ca_2GeO_4 laser. Both lasers are being developed for use in the Tissue Welding project. Preliminary data shows enhanced tissue bond strength using the Cunyite laser over other available comparable-power light sources. This result may be due to the unique tuning range in the H_2O absorption band.

D. Education, Training, Outreach, and Dissemination

Education, Training, Outreach, and Dissemination
Staff: P.-P. Ho (CCNY)
Period: 1998-2002

The goal was to provide hands-on photonic training, workshops and seminars for medical professionals and medi-photonic education and research opportunities to CCNY students. Two series of lectures were offered by the Center with the goal of increasing dialogue and information exchange between medical researchers and optical scientists and informing both about the basics of the two fields. The classes were cooperatively developed and sponsored by City College of NY, Memorial Sloan Kettering Cancer Center and the New York Eye and Ear Infirmary.

The schedules of lectures from the two series run by the Center (99-00 and 00-01) are attached. The purpose of the first series ("Introduction to Photonics for Medical Research") was to introduce medical researchers and clinicians to optics and photonics terminology and to educate

them about applications to existing and potential research instruments and clinical devices. The second series ("Introduction to the Concepts and Methods in Biomedicine") aimed to educate physics and engineering researchers about the terminology and basics of Biomedicine.

At least one doctorate was awarded directly as a result of graduate student work on this project. After receiving her degree, Dr. Yici Guo was hired by AT&T, where she is still employed.

E. Center Deliverables

Publications

1. A. Gilerson, I. Zeylikovich, and R.R. Alfano, *High Speed Grating-Generated Electronic Coherence Microscopy of Biological Tissue Without Moving Parts*, Proceedings of Coherence Domain Optical Methods in Biomedical Science and Clinical Applications (SPIE) v.3598, pp.213-215, 1999.

2. A. Katz, R.R. Alfano, A.D. Kofinas, D.A. Kofinas, P.G. Stubblefield, W. Rosenfeld, D. Beyer, D. Maulik, M.R. Stankovic, *Brain Perfusion Monitoring with Frequency-domain and Continuous-wave Near-infrared Spectroscopy: A Cross-correlation Study in Newborn Piglets*, Phys. Med. Biol., 45 (2000), 3143-3158.

3. A. J. Spielman, G. Zhang, C.-M. Yang, P. D'Ambrosio, S. Serizawa, M. Nagata, H. von Gizycki, R. R. Alfano, *Intracerebral Hemodynamics Probed by Near Infrared Spectroscopy in the Transition between Wakefulness and* Sleep, Brain Research, 866 (2000), 313-325.

4. B. Xu, J. M. Evans, V. Petricevic, S. P. Guo, O. Maximov, M. C. Tamargo, and R. R. Alfano, *Continuous-Wave and Passively Mode-Locked Operation of a Cunyite (Cr4+:Ca2GeO4) Laser*, Appl. Optics **39**,4975-4978 (2000).

5. F. Liu, J. Ying, R. Alfano, *Nonlinear Optical Signals in Active Highly Scattering Media*, SPIE Proc. 3597, 403, 1999.

6. F. Liu, J. Ying, R. Alfano, *Spatial Distribution and Detection of Nonlinear Optical Signals in Highly Scattering Media*, SPIE Proc. 3597, 403, 1999.

7. F. Liu, R. Alfano, H. Savage and S. Schantz, *Subsurface Tumor Evaluation Using Noninvasive Optical Second Harmonic Imaging*, 5th Research Workshop on the Biology, Prevention and Treatment of Head and Neck Cancer 8/98.

8. G. Zhang, S. Demos, R. R. Alfano, *Far-red and NIR Spectral Wing Emission from Tissues under 532 and 632 nm Photo-excitation*, Lasers in the Life Sciences 9, 1, 1999

9. I. Zeylikovich and R. Alfano, *Novel Grating-Generated Heterodyne Interferometric Tomography of Tissue*, Proceedings of the Conference on Optical Biopsy II, SPIE v. 3250, pp. 196-200 (1998).

10. I. Zeylikovich and R. Alfano, *Three-Exposure Interferometric Histological Image Reconstruction of Biological Tissue* Proceedings of the Conference on Optical Biopsy II, SPIE v. 3250, pp. 191-195 (1998).

11. I. Zeylikovich, A. Gilerson, R. Alfano, *Non-mechanical Grating-Generated Scanning Coherence Microscopy*, Opt. Lett. 23, 1797, 1998.

12. J. Evans, V. Petricevic, A. Bykov, A. Delgado and R. Alfano *Continuous-Wave All-Solid-State Operation of Cr^{4+}:Forsterite and Cr^{4+}:Ca_2GeO_4*, OSA Trends in Optics and Photonics Volume 17 DPSS (Diode Pumped Solid State) Lasers: Applications and Issues, ed M. Dowley, OSA, Washington DC (1998)

13. J. Tang, F. Zeng, P.P. Ho, H.E. Savage, R.R. Alfano, *Thermal Damage in Tissue Probe in Situ by Collagen Fluorescence Spectroscopic Imaging*, Lasers in Surgery and Medicine 2000; 27.

14. J. Tang, J. Evans, P. Petricevic, P.P. Ho, R.R. Alfano, *Tissue Welding Using Near-Infrared Forsterite and Cunyite Tunable Lasers.* IEEE Journal of Selected Topics in Quantum Electronic 1999, 5:1103-1106.

15. J. Tang, Zeng F., Evans J., Xu B., Savage H., Ho P.P., Alfano R.R., *A Comparison of Cunyite and Fosterite NIR Tunable Laser Tissue Welding Using Native Collagen Fluorescence Imaging,* Clinical Laser Medicine & Surgery 2000; 18.

16. J. Tang, Zeng F., Ho P.P., Alfano R.R., *Fluorescence Spectroscopic Imaging To Detect Changes in Collagen and Elastin Following Laser Tissue Welding*, Journal of Clinical Laser Medicine & Surgery 2000; 18:117-123.

17. J. Ying, F. Liu, R. Alfano, *Spatial Distribution of Two-Photon-Excited Fluorescence in Scattering Media*, Appl. Opt., 38, 224, 1999.

18. Katz, E. F. Kruger, G. Minko, C. H. Liu, R. B. Rosen, and R. R. Alfano, *Detection of Glutamate in the Eye by Raman Spectroscopy.* In: Optical Devices and Diagnostics in Materials Science, San Diego, CA, July 31 - August 4, 2000, pp. 169-171.

19. Xu, M. Lax, R. R. Alfano, *Time-Resolved Fourier Optical Diffuse Tomography,* J. Opt. Soc. Am. A 18, 1535 (2001).

20. Xu, S. K. Gayen, W. Cai, M. E. Zevallos, M. Lax, and R. R. Alfano, *Time-Sliced Three-Dimensional Inverse Image Reconstruction of Objects in Highly Scattering Media.* SPIE Proceedings on Optical Tomography and Spectroscopy of Tissue III Vol. 3597, 2 (1999).

21. Xu, W. Cai, M. Lax and R.R. Alfano, *A Photon Transport Forward Model for Imaging in Turbid Media*, Opt. Lett. 26, 1066 (2001).

22. M. Xu, W. Cai, M. Lax and R.R. Alfano, *Photon Migration in Turbid Media Using a Cumulant Approximation to Radiative Transfer*, Phys. Rev. E (submitted for publication 2002).

23. M. Xu, W. Cai, M. Lax and R.R. Alfano, *Stochastic View of Photon Migration in Turbid Media,* Phys. Rev. Lett. (submitted for publication 2002).

24. R. R. Alfano, S. G. Demos, P. Galland, S. K. Gayen, Yici Guo, P. P. Ho, X. Liang, F. Liu, L. Wang, Q. Z. Wang, and W. B. Wang, *Time-Resolved and Nonlinear Optical Imaging for Medical Applications,* Annuals of the New York Academy of Sciences, Vol.838, *Advanced in Optical Biopsy and Optical Mammography,* 145-28 (1998).

25. R. R. Alfano, S. K. Gayen, B. B. Das, M. E. Zevallos, *Time-Sliced Transillumination Imaging of Normal and Cancerous Breast Tissues*, R, OSA TOPS, 21, 36, 1998

26. S. G. Demos, H.B. Radousky and R.R. Alfano *Subsurface Imaging Using the Spectral Polarization Difference Technique and NIR Illumination*, Optical Tomography and Spectroscopy of Tissue III, B. Chance and R.R. Alfano, Eds., SPIE 3597, 406-410,1999.

27. S. G. Demos, H. B. Radousky, R. R. Alfano, *A Prototype Instrument for Subsurface Imaging in A Clinical Environment*, 3917-10, *Photonics West, BIOS 2000, Optical Biopsy III*, San Jose, California, January 22-28, 2000.

28. S. G. Demos, M. Staggs, H.B. Radousky, R.R. Alfano, *Instrumentation for Subsurface Imaging in a Clinical Environment*, R.R. Alfano, Ed., SPIE, 3917, 62-66, 2000.

29. S. G. Demos, R. R. Alfano and H. B. Radousky, *Deep Subsurface Imaging in Tissues Using Spectral and Polarization Filtering,* Optics Express, 7, 23-28, 2000.

30. S. G. Demos, W. B. Wang, J. Ali, and R. R. Alfano, *Imaging Object Hidden in Scattering Media Using Fluorescence Polarization Preservation of Contrast Agents,* Appl. Optics, 37, 792-797 (1998).

31. S. G. Demos, W. B. Wang, J. Ali, and R. R. Alfano, *New Optical Difference Approaches for Sub-Surface Imaging of Tissues*, OSA TOPS Vol.21, in *Advances in Optical Imaging and Photon Migration*, edited by J. G. Fugimoto and M. S. Patterson, 405-410 (1998).

32. S. K. Gayen, M. Alrubaiee, H.E. Savage, S.P. Schantz, and R.R. Alfano, *Parotid Gland and Tissues Investigated by Picosecond Time-Gated and Optical Spectroscopic Techniques*, IEEE J. Select. Topics Quantum Electron. 7, (2001).

33. S. K. Gayen, M. Alrubaiee, M.E. Zevallos, and R.R. Alfano, *Temporally and Spectrally Resolved Optical Imaging of Normal and Cancerous Human Breast Tissues,* Proceedings of the Inter-Institute Workshop on in vivo Optical Imaging at the NIH, Amir H. Gandjbakhche, ed. (Optical Society of America, Washington, DC, 2000), pp.142-147.

34. S. K. Gayen, M. E. Zevallos, and R. R. Alfano, *Near-Infrared Laser Spectroscopic Imaging: A Step Towards Diagnostic Optical Imaging of Human Tissues,* Lasers in the Life Sciences 8, 187 (1999).

35. S. K. Gayen, R. Alfano, *Sensing Lesions in Tissues with Light*, Opt. Express, 4, 475, 1999.

36. W. B. Wang, J. H. Ali, J. H. Vitenson, J. M. Lombardo, and R. R. Alfano, *Optical Imaging of Human Prostate Tissues,* presented in 2000 SUE Annual Meeting, Apr. 29-30, 2000, Atlanta, GA.

37. W. B. Wang, J. H. Ali, J. H. Vitenson, J. M. Lombardo, and R. R. Alfano, *Spectral Polarization Imaging of Human Prostate Tissues,* in Proceedings of "Optical Biopsy III", SPIE, Vol. 3917, 75 (2000).

38. W. B. Wang, J. H. Ali, R. B. Dorshow, M. A. Mcloughlin, and R. R. Alfano, *Time-Resolved Fluorescence Polarization Dynamics and Imaging of Fluorescein Dye Attached to Different Molecular Weight Chains*, SPIE, Vol.3600, 227-229 (1999).

39. W. B. Wang, J. H. Ali, R. B. Dorshow, M. A. Mcloughlin, and R. R. Alfano, *Temporal Polarization Dynamics and Imaging of Fluorescein Attached To Different Molecular*

Weight Chains, presented at the 1998 OSA Annual Meeting, October 4-9, 1998, Baltimore, Maryland.

40. W. B. Wang, S. G. Demos, J. Ali, G. Zhang, and R. R. Alfano, *Enhancement of Contrast Agent Images Using Spectral Fluorescence Difference*, OSA TOPS Vol.22, in *Biomedical Optical Spectroscopy and Diagnostics/Therapeutic Laser Applications,* edited by E. M. Sevick-Muraca, J. A. Izatt, and M. N. Ediger, 57-60, (1998).

41. W. B. Wang, S. G. Demos, J. Ali, G. Zhang, and R. R. Alfano, *Enhancement of Visibility of Fluorescent Object Hidden in Animal Tissue Using Spectral Fluorescence Difference Method,* Optics Comm., 147, 11-15 (1998).

42. W. Cai, M. Lax, R. Alfano, *Accurate Analytical Solution of the Photon Transport Equation in an Infinite Uniform Turbid Medium,* SPIE Proc. 3597, 15, 1999

43. W. Cai, M. Lax, and R. R. Alfano, *Analytical Solution of the Elastic Boltzmann Transport Equation in an Infinite Uniform Medium Using Cumulant Expansion*, J. Phys. Chem. B 104, 3996 (2000).

44. W. Cai, M. Lax, and R.R. Alfano, *Analytical Solution of the Polarized Photon Transport Equation in an Infinite Uniform Medium Using Cumulant Expansion,* Phys. Rev. E 63, 016606 (2001).

45. W. Cai, M. Lax, and R. R. Alfano, *Cumulant Solution of the Elastic Boltzmann Transport Equation in An Infinite Uniform Medium*, Phys. Rev. E 61, 3871 (2000).

46. W. Cai, M. Lax, R. Alfano, *Time-Resolved Optical Backscattering Model from Highly Scattering Media*, OSA TOPS, 21, 53, 1998.

47. W. Cai, S. Gayen, M. Lax, M. Xu, R. Alfano, *Inverse Reconstruction of Three-Dimensional Tomographic Images of Objects in Turbid Media from Time-Sliced Two-Dimensional Transmission Measurements*, OSA TOPS, 21, 138, 1998

48. Y. Guo, F. Liu, Q. Wang, H. Savage, N. Zhadin, P. Ho, S. Schantz, R. Alfano, *Nonlinear Optical Histological Spectroscopy and Imaging of Biological Tissues*, OSA TOPS 21, 411, 1998.

49. Y. Guo, F. Liu, Q. Wang, N. Zhadin, P. Ho, H. Savage, D. Harris, P. Sacks, S. Schantz, R. Alfano, *Second Harmonic and Two Photon Fluorescence Histology of Tissues*, SPIE Proc. 3250, 210, 1998.

50. Y. Guo, H. Savage, F. Liu, S. Schantz, R. Alfano, *Subsurface Tumor Progression Investigated by Noninvasive Optical Second Harmonic Tomography*, SPIE Proc. 3597, 480, 1999.

51. Y. Guo, P. Ho, F. Liu, Q. Wang, R. Alfano, *Noninvasive Two-Photon Excitation Imaging of Tryptophan Distribution in Highly Scattering Biological Tissues*, Opt. Comm. 154, 383, 1998

52. Y. Yang, E. Celmer, J. Koutcher, R. Alfano, *Excitation Spectroscopy Reveals Changes of Proteins and the Degree of Invasion in Malignant Tissues*, SPIE Proc. 3597, 511, 1999

Presentations

1. Conducted *Roundtable on Issues of Breast Cancer Screening* with Dr. Sanchez (Chief of Pathology) at Englewood Hospital 1/98.
2. Conducted *Roundtable on Brain Imaging* with Dr. Sassaroli (Head of Cell Imaging) at Mount Sinai Medical Center 4/98.
3. Presented 18-Lecture Series by CLICD staff *Learning the Ropes of Photonics Research* to *Students and Staff* at CCNY, sum. 1998.
4. Showed *9-videotape MIT Series Understanding Lasers and Applications* for new CCNY students, sum. 1998.
5. Offered seminar *Advances in Laser Physics, Biology, and Medical Research* for researchers and students at CCNY 7/98.
6. J. Evans, V. Petricevic and R. Alfano, Q. Fu, *Femtosecond Cr:forsterite Regenerative Amplifier* OSA Annual Meeting 10/98.
7. J. Evans, V. Petricevic, A. Bykov and R. Alfano, *Fiber-Laser-Pumped* Cr^{4+}*:*Ca_2GeO_4 *Laser*, Conference on Lasers and Electro-Optics 5/98.
8. J. Evans, V. Petricevic, R. Alfano *Supercontinuum Generation Using 1.25 μm Seed Pulses*, OSA Annual Meeting 10/98.
9. S. G. Demos, H. B. Radousky and R.R. Alfano, *Subsurface Spectral Polarization Imaging Using NIR Laser Illumination*, Optical Society of America, 1998 Annual Meeting, TuZ2, Baltimore, Maryland, October 4-9, 1998.
10. S. G. Demos, H. B. Radousky, R. R. Alfano, *A Prototype Instrument for Subsurface Imaging in a Clinical Environment*, 3917-10, Photonics West, BIOS 2000, Optical Biopsy III, San Jose, California, January 22-28, 2000.
11. S. G. Demos, H.B. Radousky and R. R. Alfano *Subsurface Imaging Using The Spectral Polarization Difference Technique and NIR Illumination*, 3597-46, Optical Tomography and Spectroscopy of Tissue III, BIOS '99, San Jose, California, 23-29 January 1999.
12. S. G. Demos, W.B. Wang, J. Ali, and R. R. Alfano, *New Optical Difference Approaches for Sub-Surface Imaging of Tissues*, presented in OSA 1998 Topic Meeting, March 8-11, 1998, Orlando, FL.
13. S. Gayen, M. Zevallos, and R. Alfano, *Time-Sliced and Spectroscopic Near-Infrared Imaging of Normal and Cancerous Human Breast Tissues*, OSA Annual Meeting 10/98.
14. Y. Guo, F. Liu, Q. Wang, H. Savage, N. Zhadin, P. Ho, S. Schantz and R. Alfano, *Nonlinear Optical Histological Spectroscopy and Imaging of Biological Tissues*, OSA Topical Meeting 3/98.
15. Y. Guo, F. Liu, Q. Wang, N. Zhadin, P. Ho, H. Savage, D. Harris, P. Sacks, S. Schantz, and R. Alfano, *Second Harmonic and Two Photon Fluorescence Histology of Tissues*, Photonics West, Bio '98 1/98.
16. Y. Guo, H. Savage, F. Liu, S. Schantz, P. Ho and R. Alfano, *Optical Second Harmonic Tomography of Subsurface Tumor Evaluation*, OSA Annual Meeting 10/98.
17. A. Katz, E. F. Kruger, G. Minko, C-H Liu, R. B. Rosen, R. R. Alfano, *Detection of Glutamate in the Eye by Raman Spectroscopy* (Submitted J. Biomed. Optics 2002).

18. A. Katz, T. K. Gayen, G. Minko, H. E. Savage, A. Alimova, S. A. McCormick, R. R. Alfano, *Noninvasive Spectroscopic Analysis of Laser Welded Aorta and Skin Tissues*, in SPIE's International Symposium (BIOS 2002), 19 – 25 January, San Jose, California.

19. F. Liu, Coherence Grating-Generated Tomography with Novel Axial Scanning Line, *Subsurface Structure Mapping of Biological Tissues Using Second Harmonic and Two Photon Fluorescence by Ultrashort Laser Pulses*, OSA Annual Meeting, invited paper 10/98.

20. W. B. Wang, J. H. Ali, J. H. Vitenson, J. M. Lombardo, and R. R. Alfano, *Spectral Polarization Imaging Study on Human Prostate Tissues,* presented at the SPIE BIOS 2000 Meeting in January 23-24, 2000, San Jose, CA.

21. W. B. Wang, J. H. Ali, J.H. Vitenson, J.M. Lombardo, and R.R. Alfano, *Optical Imaging of Human Prostate Tissues,* presented in 2000 SUE Annual Meeting, April 29-30, 2000, Atlanta, Georgia.

22. W. B. Wang, J. H. Ali, R. B. Dorshow, M. A. McLoughlin, and R. R. Alfano *Time-Resolved Fluorescence Polarization Dynamics of Fluorescein Dye Attached to Different Molecular Weight Chains and their Images in Turbid Media,* presented at the SPIE 1999 Biomedical Optics Meeting, January 23-29, 1999, San Jose, California.

23. W. B. Wang, J. H. Ali, R. B. Dorshow, M. A. McLoughlin, and R. R. Alfano *Temporal Polarization Dynamics and Imaging of Fluorescein Attached to Different Molecular Weight Chains,* presented at the OSA Annual Meeting, October 4-9, 1998, Baltimore, Maryland.

24. W. B. Wang, J. H. Ali, R. R. Alfano, J. H. Vitenson, and J. M. Lombardo, *Spectral Polarization Imaging of Prostate and Rectum-Membrane-Prostate Tissues*, presented at the Symposium of "Frontiers of photonics", November 5, 2001, New York, NY.

25. W. B. Wang, S. G. Demos, J. Ali, G. Zhang, and R. R. Alfano, *Enhancement of Contrast Agent Images Using Spectral Fluorescence Difference,* presented in OSA 1998 Topic Meeting, March 8-11, 1998, Orlando, FL.

26. W. Wang, S. Demos, J. Ali, G. Zhang and R. Alfano, *Enhancement of Visibility of Objects Hidden Inside Tissues Using Spectral Fluorescence Difference Imaging Technique*, OSA Topic Meeting 3/98.

27. Y. Yang, E. J. Celmer, J. A. Koutcher, and R. R. Alfano, *DNA and Protein Changes in Tissues Probed by Kubelka Munk Spectral Function*. Optical Biopsy III. Vol. 3917 January 24-28, 2000 San Jose, Cal.

28. Y. Yang, E. J. Celmer, J. A. Koutcher, and R. R. Alfano, *Excitation Spectroscopy Reveals Changes of Proteins and the Degree of Invasion in Malignant Tissues*. Proc. Optical Tomography and Spectroscopy of Tissue III. Vol. 3597 January 24-28, 1999 San Jose, Cal.

29. Y. Yang, E. J. Celmer, J.A. Koutcher, P. P. Ho, and R. R. Alfano, *DNA and Protein Change in Human Breast Tissues by Diffuse Relfectance Spectrum*. Optical Sensing, Imaging and Manipulation for Biological and Biomedical Applications. Vol. 4082 July 26-28, 2000 Taipei, Taiwan.

30. J. Ying, F. Liu and R. Alfano, *Spatial Distribution of Two-Photon Excited Fluorescence in Scattering Media*, OSA Annual Meeting 10/98.

31. H. Zhang, A. Spielman, P. D'Ambroso, M. Nagato, S. Serizawa, D. Conroy, G. Lombardo, and R. R. Alfano, *Near-infrared Spectroscopy (NIRS) Measurement of Cerebral Hemodynamics Parallel Finger Pulse Oximetry in Obstructive Apnea*, 1999 Annual Meeting of Association of Professional Sleep Societies, Orlando, Florida (1999).

32. G. Zhang, T. K. Gayen, A. Katz, Y. Budansky, J. Evans, X. Shixiang, H. E. Savage, S.A. McCormick, R.R. Alfano, *Near-Infrared Welding of Aorta and Skin Tissues Using a Cr4+: YAG Laser*, SPIE's International Symposium (BIOS 2002), 19 – 25 January, San Jose, CA.

Patents

U.S. Patents

1. R. R. Alfano and W. B. Wang, *Method and System for Examining Biological Materials Using Low Power CW Excitation Raman Spectroscopy,* No. 6,151,522, Nov. 21, 2000.

U.S. Patent Applications

1. R. R. Alfano, J. Tang, J. Evans, P.P. Ho, U.S. provisional patent application number 60/282,827, entitled *Gelatin Based and Power-Gel© as Solders for Cr^{4+} Laser Tissue Welding and Sealing of Lung Air Leak and Fistulas in Organs*, was filed April 10, 2001. Subsequently, a U.S. Patent Application, number 10/119,914, was filed 04/10/2002.

2. R. R. Alfano, J. Tang, J. Evans, P.P. Ho., U.S. patent application number 09/767,129, entitled *Fluorescence Spectroscopic Imaging to Improve the Techniques of Laser Tissue Welding, Burn Diagnosis and Other Laser Surgical Therapies* was filed 01/22/2001.

3. R. R. Alfano, J. Tang, and P-P Ho, U.S. patent application number 09/767,125, entitled *System and Method of Fluorescence Spectroscopic Imaging for Characterization and Monitoring of Tissue Damage*, was filed 01/22/2001.

4. R. R. Alfano, S. G. Demos and G. Zhang, U.S. patent application number 09/493,939, entitled *Method and Apparatus for Examining a Tissue Using the Spectral Wing Emission therefrom Induced by Visible to Infrared Photoexcitation*, was filed 01/28/2000.

5. R. R. Alfano, W. B. Wang, (continuation in part) U.S. patent application number 09/717,327, entitled *Method and System for Examining Biological Materials Using Low Power CW Excitation Raman Spectroscopy*, was filed, 10/20/2000.

6. R. R. Alfano, Y. Yang, U.S. patent application number 09/598,007, entitled *Technique for Examining Biological Materials Using Diffuse Reflectance Spectroscopy and the Kubelka-Munk Function*, was filed 6/19/2000.