# AMERICAN SOCIETY FOR LASER MEDICINE AND SURGERY TWENTY-SIXTH ANNUAL MEETING BOSTON, MASSACHUSETTS APRIL 5–APRIL 9, 2006 ABSTRACTS

## BASIC SCIENCE: THE CUTTING EDGE IN OPTICAL DIAGNOSTICS AND THERAPEUTICS OF TISSUES AND CELLS

### 1

**COLLAGEN-SUGAR ALCOHOL INTERACTIONS IN SKIN TISSUE OPTICAL CLEARING**

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**Background and Objectives:** Light based diagnostic and therapeutic techniques are restricted due to the turbidity of skin. The scattering associated with collagen limits the amount of photons delivered to the embedded target. Sugar alcohols have the ability to reduce skin turbidity by perturbing the collagen structure. This study examines the interaction between collagen and a series of sugar alcohols in order to understand the mechanism of skin tissue clearing.
**Study Design/Materials and Methods:** The inhibiting effect of a series of sugar alcohols on fibrillogenesis was measured in a collagen solubility study. The sugar alcohols were applied to skin tissue and changes in optical properties were measured using an integrating sphere method.
**Results:** The inhibiting effects of sugar alcohols on collagen fibrillogenesis were found to be dependent on chain length and hydroxyl group placement. Fibrillogenesis inhibition increased with increasing chain length. Comparison studies using diols suggest the importance of steric interactions in collagen fibrillogenesis inhibition. Correlations were examined between collagen fibrillogenesis inhibition and tissue optical clearing.
**Conclusion:** The hydroxyl groups of the sugar alcohols interfere with hydrogen bonding between collagen triple helices. This interaction results in limiting collagen's ability to form fibers as well as the perturbation of collagen structure seen in the optical clearing of skin tissue.



Published online in Wiley InterScience (www.interscience.wiley.com).
DOI 10.1002/lsm.20312

## 2

### EFFECT OF OPTICAL CLEARING AGENTS ON THE OPTICAL PROPERTIES OF SQUAMOUS EPITHELIAL TISSUE

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**Background and Objectives:** Optical Clearing Agents (OCAs) have been shown to increase depth penetration within turbid tissue. OCAs may be advantageous for light-based diagnostic techniques for epithelial pre-cancer detection. Previous studies have quantified the effects of OCAs on tissue optical properties *in vitro*. This study quantified the effect of two commonly used OCAs, glycerol and dimethyl sulfoxide (DMSO) on the absorption and scattering properties of the *in vivo* hamster cheek pouch, which is a model of stratified squamous epithelial tissue.
**Study Design/Materials and Methods:** Diffuse reflectance spectra between 400–460 nm were obtained from both cheeks of 12 hamsters before and after immersion in DMSO, glycerol or a phosphate buffer saline (PBS) control for 20 minutes. A Monte Carlo model of diffuse reflectance was utilized to derive the reduced scattering coefficient, hemoglobin concentration and total hemoglobin saturation of the tissue.
**Results:** DMSO significantly reduces the reduced scattering coefficient by 12.54 $cm^{-1}$, the total hemoglobin concentration by 15% and hemoglobin saturation by 77.48 μM. Glycerol significantly increased the total hemoglobin content by 116.11 μM. Glycerol also decreased the reduced scattering coefficient by 5.81 $cm^{-1}$, but this value was not statistically significant.
**Conclusions:** DMSO and glycerol act upon tissue in different ways shown by the change in the optical properties. This variability suggests that OCAs should be investigated separately for a given target tissue.

## 3

### EFFECT OF OPTICAL CLEARING AGENTS ON THE MORPHOLOGY OF SQUAMOUS EPITHELIAL TISSUES

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**Background and Objectives:** Hyperosmotic agents are chemical substances that allegedly "optically clear" tissues by causing a decrease in the refractive index mismatch between the scatterers in the tissue and the surrounding medium. This study analyzes the effect of the optical clearing agents dimethyl sulfoxide (DMSO) and glycerol on the morphologic features of squamous epithelial tissues in the hamster cheek pouch using multiphoton laser scanning microscopy.
**Study Design/Materials and Methods:** In this study, three male Golden Syrian hamsters were used for each agent. After imaging the untreated tissue, the optical clearing agent was applied for 20 minutes and a new set of images were taken. Changes in morphology were quantified using the images.
**Results:** Morphologic changes of the tissue are dependent on the agent used. Some of the changes caused by DMSO include: a loss of the stratified structure of the cells in the epithelial layer and the destruction of the epithelial cell membranes. The application of glycerol caused a decrease in the epithelial thickness, a loss of the stratified structure, and the dissociation of the collagen fibers.
**Conclusions:** The effect of optical clearing agents on tissue constituents vary and thus must be treated separately. The tissue shrinkage caused by these optical clearing agents could be beneficial in light-based techniques for the diagnosis and management of pathologies.

## 4

### CONTROLLING SPECTRAL PROPERTIES OF SKIN WITH OPTICAL CLEARING

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**Background and Objective:** Previously we have proposed a method of enhancement of epidermal permeability *via* creating a lattice of islands of damage (LID) in the stratum corneum (SC) to provide more effective action of optical clearing agents (OCA). The goal of this study was to compare the efficacy of optical clearing attainable at different wavelengths and measure the effect of optical clearing on the skin autofluorecence.
**Materials and Methods:** Pig skin was used for *ex vivo* measurements, and human skin was used for both *ex vivo* and *in vivo* experiments. A flashlamp EsteLux system (Palomar Medical Technologies, Inc.) (10–20 ms, 650–1200 nm, and 9–27 $J/cm^2$) including a mask providing patterned light delivery (center size ~75–120 μm, lattice pitch ~450–500 μm) was used to create the LID. Various OCA were applied. Measurements of transmittance and autofluorecence spectra were conducted.
**Results:** In the visible and near IR spectral ranges the efficiency of clearing differed 1.5–2.5 times depending on wavelength. Realistic Monte Carlo simulations of skin spectral properties correlated well with the experimental spectra and their dynamics after OCA applications.
**Conclusion:** Enhancement of skin permeability using LID proved to be an effective technique for delivery of OCA into skin. Changes in the skin autofluorecence signal were consistent with the hypothesis of modifying spectral filtration properties of skin by immersion optical clearing.

## 5

### IMPROVEMENT OF PORT WINE STAIN LASER THERAPY THROUGH MULTIPLE-INTERMITTENT CRYOGEN SPURTS AND TWO-WAVELENGTH LASER PULSES

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**Background and Objectives:** Presently, all port wine stain (PWS) patients are treated using single cryogen spurt and single laser pulse exposure, which does not produce complete lesion blanching in the vast majority of patients. In this study, the feasibility of applying multiple cryogen spurts intermittently with multiple two-wavelength laser pulse exposures (MCS-MTWLP) is studied numerically.
**Study Design/Materials and Methods:** Three treatment procedures were selected: 1) cryogen spurt + 585 nm laser pulse; 2) cryogen spurt + 1064 nm laser pulse + cryogen spurt + 585 nm laser pulse; 3) cryogen spurt + 1064 nm laser pulse + cryogen spurt + 532 nm laser pulse. The treatment procedure was simulated with Monte Carlo light distribution and finite element heat diffusion models. Possible epidermal damage and photocoagulation of PWS blood vessels of various diameters (50–150 μm) were calculated with an Arrhenius-type kinetic model.
**Results:** The results show that the proposed MCS-MTWLP approach can provide sufficient epidermal protection while at the same time achieving higher core intravascular temperatures over longer periods of time. The second procedure produces largest coagulation area when the PWS blood vessel diameter is greater than 100 μm.
**Conclusions:** The MCS-MTWLP approach is promising to improve the therapeutic outcome for patients with large PWS blood vessels.

## 6

### NUMERICAL MODELING OF CRYOGEN SPRAY COOLING-ASSISTED LASER SURGERY AT HYPOBARIC PRESSURE FOR TREATMENT OF PORT WINE STAINS

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**Background and Objectives:** Port wine stain (PWS) birthmarks respond favorably to 585 nm pulsed-dye laser treatment, but complete removal of small diameter vessels is rarely achieved. The objective of the present numerical study is to assess the effect of applying local hypobaric pressure, during PWS treatment with cryogen spray cooling-assisted laser surgery, on the thermal damage induced to small vessels.
**Study Design/Materials and Methods:** Mathematical models of mechanical deformation, light deposition, heat diffusion and failure acceleration are used to compute elongation, absorbed energy, temperature and thermal damage distributions of skin and blood vessels subject to laser surgery under atmospheric and hypobaric pressure conditions.
**Results:** Under atmospheric pressures, the laser energy deposited in small diameter vessels (~5–15 μm) is insufficient to induce irreversible thermal damage. Under hypobaric pressures (~15 in Hg), there is an increase in blood volume fraction due to vessel elongation (~15 %) that allows depositing more energy in these small vessels, such that irreversible thermal damage is successfully induced.
**Conclusions:** Local hypobaric pressure on PWS skin induces dilation of blood vessels and, consequently, increases their blood volume fraction. As a result, enough laser energy may be delivered to small resilient PWS vessels removing them completely improving lesion blanching.

## 7

### A COMPARATIVE STUDY OF CRYOGEN SPRAY COOLING WITH R-134A AND R-404A: IMPLICATIONS FOR LASER TREATMENT OF DARK HUMAN SKIN

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**Background and Objective:** Cryogen spray cooling (CSC) with R-134a (boiling point≈−26.2°C at 1 atm) has shown poor efficacy in protecting dark human skin during cutaneous laser treatment. We investigated the potential of CSC with R-404a (boiling point≈−46.5°C at 1 atm) for improved epidermal protection in dark human skin at three levels: *in-vitro*, *ex-vivo*, and *in-vivo*.
**Study Design/Materials and Methods:** A skin phantom was used to estimate CSC-induced heat removal from the phantom. Normal *ex-vivo* Fitzpatric types V-VI human skin samples were used to investigate the thermal response of skin epidermis to CSC in conjunction with 595-nm laser irradiation. *In-vivo* rabbit ear vasculature was used to assess the influences of CSC on the photothermolysis of dermal vasculatur.
**Results:** For a spurt duration $\tau_{csc} = 300$ ms, CSC with R-404a increased the heat removal from skin phantom by approximately 11% in comparison to that with R-134a, and increased the threshold radiant exposures for irreversible epidermal damage by 3 J/cm$^2$ in *ex-vivo* dark skin. CSC using R404-a with $\tau_{csc} <= 300$ ms did not induce morphological changes to human skin. CSC (R-134a or R-404a) with $\tau_{csc} = 100–300$ ms increased the most superficial location of thermally-damaged dermal vasculature compared with the sites without CSC.
**Conclusions:** CSC with R-404a can improve epidermal protection, and is safe in terms of CSC-induced injury to human skin when $\tau_{csc} <= 300$ ms. Non-specific cooling of superficial dermal vasculature may occur for $\tau_{csc} = 100–300$ ms.

## 8

### EFFECTIVE INFRARED ABSORPTION COEFFICIENT FOR PHOTOTHERMAL RADIOMETRIC MEASUREMENTS IN BIOLOGICAL TISSUES

**Boris Majaron and Matija Milanič**

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**Background and Objective:** Pulsed photothermal radiometry (PPTR) involves time-resolved acquisition of infrared (IR) radiant emission from laser-irradiated samples, to enable temperature depth profiling and three-dimensional imaging of chromophores in optically scattering biological tissues. We address the issue of optimal reconstruction of laser-induced temperature profile from broad-band radiometric signals within the commonly applied monochromatic approximation.

**Study Design/Materials and Methods:** Starting from a few analytical temperature profiles ("objects"), resembling those expected in dermatologic vascular lesions, we computed realistic PPTR signals for a number of spectral acquisition bands, taking into account the influence of spectral variation of the IR absorption coefficient in human skin and spectral sensitivity of a typical InSb radiation detector. Then, we applied a novel code for reconstruction of the initial temperature profiles on a personal computer. Through quantitative analysis of the mismatch between the reconstructed temperature profiles ("images") and objects, we determined optimal effective IR absorption coefficient for each case under study.

**Results:** Under typical experimental conditions, the monochromatic approximation in PPTR signal analysis yields significant artifacts in the images, especially with more complex objects. The optimal effective value of the IR absorption coefficient varies strongly with the selected spectral acquisition band and characteristic depth of the object.

**Conclusion:** In PPTR temperature depth profiling of biological tissues, it is worthwhile to determine the optimal value of the IR absorption coefficient and, if possible, select the most appropriate spectral acquisition band. For profiling and imaging of more complex structures, however, the monochromatic approximation may have to be abandoned.

## 9

### SELECTION OF OPTIMAL IR DETECTOR FOR PPTR OF PORT WINE STAIN LESIONS

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**Background and Objective:** Pulsed phototermal radiomtry (PPTR) can be used for non-invasive depth profiling of port wine stain (PWS) birthmarks, aimed towards optimizing laser therapy on an individual patient basis. Selection of infrared detector is a key consideration in designing an experimental setup for PPTR.

**Study Design/Materials and Methods:** We studied PPTR of numerical PWS models in computer simulations. Different PWS models were used, from single layer to Monte Carlo model of multilayer PWS. We considered three detectors with different spectral responses (InSb 3.0 μm–5.6 μm; HgCdTe 3.0 μm–12.0 μm; HgCdTe 8.0 μm–14.0 μm) over multiple acquisition bands. We added realistic levels of noise composed of detector noise and background noise for a chosen acquisition band. We reconstructed image of PWS depth profile from simulated signal and calculated reconstruction error for a certain detector and acquisition band. We also performed measurements on tissue phantoms and compared experimental results to those obtained from simulations.

**Results:** Simulation results indicate that optimal results for the InSb detector are achieved, when acquisition band 4.3 μm–5.6 μm is used. Second detector, MCT 3.0 μm–12.0 μm, had a slightly better performance over a wider spectral band, 7.0 μm–12.0 μm. Third detector gave the highest reconstruction error. Reconstruction results of experimental PPTR signals supported the simulation results.

**Conclusion:** For the discussed application, exploring the 6–10 μm acquisition band with a HgCdTe detector offers a slightly better performance than InSb detector used in the customary 3–5 μm band.

## 10

### THERMODYNAMIC MODELING OF SEQUENTIAL, DUAL WAVELENGTH TREATMENT OF PORT WINE BIRTHMARKS

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**Background and Objectives:** Previous investigation has shown opportunities for the use of sequential, dual-wavelength treatments, leading to the development of a 595-nm Pulse dye, 1064-nm Nd:YAG laser device for treatment vascular lesions. The opportunity to alter blood wavelength absorption creates new, dynamically changing conditions which may affect both safety and clinical efficacy. In an effort to better understand these conditions, we developed a model to assess the impacts of these changing conditions on various geometries of vascular lesions.

**Study Design/Materials and Methods:** A laser-tissue interaction model was constructed to model the case of a 6 $j/cm^2$, 10-msec, 595-nm laser pulse; a delay of 500–1000 msec, a 60 $j/cm^2$ Nd:YAG pulse, in the presence of cold air cooling, and where the initial pulse causes 0–100% conversion of blood hemoglobin to Meth-Hemoglobin. The tissue models assume a variety of lesion geometries, including telangiectasia; light (5% blood fraction), dark (10% blood fraction) and blebbed (to 4-mm thickness) Port Wine Birthmarks. The model reports on-axis temperature 100-msec following the final laser pulse.

**Results:** The model reports a significant increase in lesion temperature associated with increased Meth-Hemoglobin conversion, and under some circumstances, significant increase in surface temperature which may predict epidermal damage.

**Conclusions:** modeling sequential, dual wavelength treatment of various lesions provides guidance in predicting clinical effect for a variety of lesion geometries. Specific geometries and associated lesion types will be discussed.

## 11

### THROMBOSIS AS AN INTEGRAL PART OF ENDOVASCULAR LASER-TISSUE INTERACTIONS

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**Background/Objective:** It was recently proposed that site-specific pharmaco-laser therapy (SSPLT), in which procoagulant drugs are encapsulated in a photoactivatable drug delivery system, may act as an adjuvant in enhancing selective photothermolysis-induced vascular necrosis and corollary lesional clearance. To unequivocally demonstrate that thrombosis is a direct consequence of photocoagulation, we performed intravital fluorescence microscopy on laser-irradiated hamster dorsal vessels in combination with different platelet staining techniques.
**Study Design/Materials and Methods:** Following surgical exposure of hamster skin fold venules, platelets were stained by iv injection of 5,6-carboxyfluorescein encapsulating PEGylated liposomes or fluorophore-conjugated anti-glycoprotein Ib monoclonal antibodies (Mabs). The activation state of platelets was assayed by infusion of fluorescently-labeled anti-CD62P Mabs, and fibrin cross-polymerization was analyzed by administration of fluorescently-labeled anti-fibrin Mabs. Thrombosis was induced with a 532 nm frequency-doubled Nd:YAG laser, and thrombus dynamics were visualized for 30 minutes with an intravital fluorescence microscope. Lesional size was quantified as a function of time by fluorescence intensity analysis.
**Results:** Endovascular laser-tissue interactions are characterized by a photothermal response (formation of a coagulum) and a hemodynamic response (platelet aggregation). A nidus forms within one minute after laser irradiation and expands over a course of 10 minutes, after which it stabilizes and generally recedes. Thrombus development is facilitated by activated platelets and fibrin cross-polymerization.
**Conclusion:** With thrombosis as an integral part of endovascular laser-tissue interactions, different SP-based treatment modalities targeting components of the hemostatic system (such as SSPLT) could be developed to improve endoluminal occlusion and stimulate inflammatory removal of damaged ectatic vasculature.

## 12

### OPTIMIZATION OF THERMAL DEPTH PROFILING OF VASCULAR LESIONS: AUTOMATED REGULARIZATION

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**Background and Objectives:** Pulsed Photo-Thermal Radiometry (PPTR) is a non-invasive, non-contact diagnostic technique which aims at locating and quantifying cutaneous chromophores such as melanin (epidermis) and hemoglobin (vascular structures). Real-time clinical application of PPTR is limited because inversion of PPTR temporal signals into depth profiles is an inherently ill-posed problem, requiring trained user intervention to regularize the inversion solution. We studied the feasibility of automated regularization procedures for PPTR.
**Study Design:** Average vessel depths, diameters and densities, derived from histology of 30 Port Wine Stain (PWS) patients, were used to generate 50 randomized PWS geometries. A Monte Carlo algorithm and a finite differences method were used to compute corresponding light distributions and PPTR signals. Four trained individuals inverted the signals in a blinded fashion, using conventional regularization methods. Accuracy of the inverted profiles was quantified and compared with various automated regularization methods.
**Results:** A Student's t-test (paired, 2 tailed), comparing the different regularization approaches, indicated that automated regularization leads to chromophore depth profiles that are significantly more accurate ($p<0.05$) than those for conventional regularization approaches.
**Conclusions:** Similar, or better, accuracy reconstructions can be achieved with an automated regularization procedure which enhances prospects for a user friendly clinical application of PPTR to optimize laser therapy on an individual patient basis.

## 13

### A NEW MATHEMATICAL MODEL TO VISUALIZE SELECTIVE PHOTOTHERMOLYSIS OF INTENSE PULSED LIGHT SOURCES (IPL)

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**Background and Objective:** The concept of selective photothermolysis of vascular disorders led to the development of pulsed dye lasers. Various mathematical models have been developed to predict optimal lasers parameters. In contrast to lasers, the use of IPL for vascular disorders has been not investigated by such models, so far. A recently developed model was now adapted to study the vessel damage using different light parameters of IPL.
**Materials and Methods:** Due to broadband emission of the IPL, the optical parameters must be considered in the modeling at different wavelengths at the same time. The diffusion approximation was used to calculate the photon distribution in the skin for IPL. To determine the temperature in the tissue, the heat and optical diffusion equations were solved simultaneously, using the finite element method (FEM).
**Results:** For three different broadband emission spectra (500, 550 and 650 nm cut off), the calculations were performed. For the different filters different fluences are necessary to achieve a sufficient vessel temperature (at least 18 J/cm$^2$). For vessel sizes ranging from 20 to 1000 μm, the 300 μm vessel responded optimal. When changing the spectrum of IPL different results of vessel damage were achieved.
**Conclusion** The present model of light tissue interaction is a powerful tool to elucidate the IPL parameters that are necessary for optimal therapeutical outcome.

## 14

### LONG TERM STUDY OF DORSAL SKINFOLD WINDOW MODEL FOR MICROVASCULATURE THERAPIES

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**Background and Objectives:** The rodent dorsal skinfold window chamber model has been used in studies of photodynamic and photothermal therapies. In general, such studies have evaluated microvascular dynamics over relatively short periods of time (i.e., on the order of days). The objective of this study was to characterize the stability of the rodent dorsal skinfold window chamber over the course of several weeks.
**Materials and Methods:** A robust, sterile protocol involving surgical installation of a titanium window chamber on Golden Syrian hamsters was developed to permit observation of full-thickness skin from both the epidermal and subdermal sides. Laser speckle imaging was periodically used to image the exposed microvasculature and assess stability of blood flow. The window chamber was visually checked for signs of infection or hemorrhagin.
**Results:** We observed an initial period of vascular remodeling, presumably due to the wound healing response to the surgery. We identified small but resolvable issues with dehydration and animal discomfort.
**Conclusions:** The preliminary study shows that the rodent dorsal skinfold window chamber model is suitable for long–term, longitudinal studies of microvascular-targeted therapies.

## 15

### OPTIMAL PARAMETERS FOR THE TREATMENT OF LEG VEINS USING Nd:YAG LASERS AT 1064 nm

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**Background and Objective:** The treatment of large vessels such as leg veins is successfully performed in clinical practice using pulsed Nd:YAG lasers. To elucidate the governing parameters in selective photothermolysis of large vessels, a recently developed mathematical model for photothermolysis was adapted for leg veins.
**Materials and Methods:** A mathematical model calculated the temperature values during and after laser heating of vessels using a Nd:YAG at 1064 nm. The thermal damage in the vessel and the laser efficiency was calculated. The laser efficiency is defined as the ratio of the thermal damage in the targeted vessel and the applied laser fluence (J/cm$^2$).
**Results:** The calculated temperature distribution within blood vessels and dermis was in good agreement with clinical findings. The laser induced thermal damage improves with increasing vessel diameter, in agreement with clinical findings. For laser fluence of 100–400 J/cm$^2$, the pulse duration and the beam diameter have minor contribution. Excess dermis heating and pain can be reduced by using moderate fluence of 100–200 J/cm$^2$.
**Conclusion:** A lookup table for optimal treatment of leg veins is presented using Nd:YAG lasers at 1064 nm. The maximal efficiency is predicted for fluences of 100–200 J/cm$^2$ in a wide range of pulse duration (10–60 ms).

## 16

### MATHEMATICAL MODELING OF ENDOVENOUS LASER TREATMENT (EVLT)

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**Background and Objectives:** Endovenous laser treatment (EVLT) has been recently proposed to treat Great Saphenous Vein (GSV) and Small Saphenous Vein (SSV) reflux. Successful EVLT depends on the selection of optimal parameters in order to achieve an optimal vein damage while avoiding side effects. Mathematical modeling of EVLT could provide a better understanding of the EVLT process and could determine the optimal dosage as a function of vein diameter.
**Study design/Materials and Methods:** The model is based on calculations describing the light distribution using the diffusion approximation of the transport theory, the temperature rise using the bioheat equation and the dependency of thermal and optical properties on the temperature and the injury (Arrhenius damage model). The geometry to simulate EVLT was based on a 2D model consisting of a cylindrically symmetric blood vessel including a vessel wall and surrounded by an infinite homogenous tissue. The mathematical model was implemented using the Macsyma-Pdease2D software (Macsyma Inc., Arlington, MA, USA).
**Results:** Vessel wall damage for CW and single shot was calculated for different vein diameter (0.5 mm up to 5 mm) and energy ranging from 2 to 40J. The influence of the pullback distance could be considered. At last, using this mathematical modeling, it was possible to determine a linear equation for calculating the optimal energy as a function of vein diameter.
**Conclusion:** The parameters determined by mathematical modeling are in agreement with those used in clinical practice. This model could be a very useful tool to simulate EVLT.

## 17

### ENDOVASCULAR PHOTOTHERMAL RESPONSE AS FUNCTION OF BLOOD FLOW VELOCITY AND LASER PULSE DURATION

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**Background and Objective:** The clinical end-point of selective photothermolytic (SP) treatment of port wine stains (PWS) embodies significant dermal blood volume reduction by photocoagulation and inflammatory remodeling of ectatic vasculature. In SP, pulse duration and blood flow velocity play an important role. We investigated the laser-induced endovascular photothermal response as a function of blood flow velocity and pulse duration.
**Study Design/Materials and Methods:** Hamster dorsal skin fold venules (n = 95) were irradiated with a 532 nm laser at pulse durations of 30, 60, 90, 120, and 150 ms. An intravital microscope was employed to visualize iv-administered fluorescent microspheres and laser-induced endovascular photothermal responses. Thermal coagulum size, coagulum detachment, and vasoconstriction were analyzed as function of flow velocity and pulse duration.
**Results:** Both thermal coagulum size and the extent of vasoconstriction increased with pulse duration and were not influenced by blood flow velocity. Coagulum dislodgement increased with increasing blood flow velocity.
**Conclusions:** Longer pulse durations and low blood flow velocities enhance laser-induced occlusion of blood vessels. PWS-specific blood flow velocities should be quantified and clinical flow modulation techniques should be further explored in conjunction with continued efforts to safely increase pulse durations.

## 18

### CHANGES IN SKIN PROPERTIES FOLLOWING MINOR TRAUMA

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**Background and Objective:** Non-fatal minor trauma like bruises is currently evaluated by direct visual inspection or inspection of photos. In a clinical setting such subjective methods may lead to wrong conclusions regarding cause and severity of an injury. In cases of domestic violence an accurate diagnosis might be essential to determine if further legal investigations should be undertaken.
**Materials and Methods:** Controlled, minor traumatic injuries were inflicted on anesthetized domestic pigs. Reflection spectra in the 400–1700 nm wavelength range were collected from normal and bruised skin. Spectra were collected three times from each injury; before inflicting the injury, immediately after injury, and a few hours later. Biopsies were also collected from the injury sites. The experiments were approved by the regional ethical committee on animal care.
**Results:** Preliminary results show that the severity of the injuries depended strongly on object shape and speed at impact. The results indicate that a shear force was required to create hematomas if the object hit the skin with a low speed. The inflicted injuries were comparable to injuries seen in a clinical setting.
**Conclusion:** Further investigations are required to be able to fully classify minor traumatic injuries. A thorough knowledge on the relationship between object shape and impact speed is essential to solve this problem. Preliminary results indicate that this can be achieved by controlled experiments using a pig model.

## 19

### WIDE-FIELD SPATIAL MAPPING OF IN-VIVO SKIN OPTICAL PROPERTIES AND CHROMOPHORES USING MODULATED IMAGING

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**Background and Objectives:** We have developed a new imaging modality, Modulated Imaging, capable of wide-field, spatially-resolved measurement of *in-vivo* optical properties of skin. Based on spatial light modulation, the method is inexpensive, non-contact, and allows spatial mapping of tissue absorption and reduced scattering coefficients at any wavelength between 450 and 1100 nm. Moreover, control of the optical penetration depth at a given wavelength is possible through selection of illumination pattern frequency. Multi-spectral imaging further allows quantitative mapping of oxy- and deoxy-hemoglobin, melanin, and water concentrations.
**Study Design/Materials and Methods:** We have measured the spatially-varying optical properties of human forearm over a 5 cm × 3 cm field of view at wavelengths ranging from 650 nm to 1000 nm using Modulated Imaging. At each wavelength, 42 spatial frequencies were sampled, allowing probing depths ranging from sub-millimeter to > 5 mm.
**Results:** We have determined the optical properties of *in-vivo* skin and demonstrated control of interrogation volume by choice of spatial frequencies during analysis. Functional maps of melanin, hemoglobin and water concentrations, as well as tissue oxygenation and blood volume were determined from absorption spectra and reveal quantitative contrast spatially and with depth.
**Conclusions:** Spatially-resolved measurement of *in-vivo* skin optical properties and chromophores has been achieved, providing quantitative information with strong potential for laser-based therapeutic guidance.

## 20

### MULTI-SPECTRAL AND LIFETIME SPECTROSCOPY OF FLUOROPHORES IN PIGMENTED SKIN LESIONS

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**Background and Objectives:** Fluorescence spectroscopy and fluorescence lifetimes are good methods of identifying the environment of the fluorophores and can potentially be used for early melanoma detection.
**Study Design/Materials and Methods:** Components of pigmented lesions, including melanin, collagen, protoporphyrin IX, and flavin adenine dinucleotide (FAD) were investigated for fluorescence profiles and lifetimes in wet and dry environments. Major changes in the fluorescence induced by the environment change were sought.
**Results:** The fluorescence of wet sample melanin was identified to have a peak at 550 nm with a FWHM of 135 nm and at 0.365 ns lifetime. Remarkably reduced fluorescence, if any, was measured from dry melanin. Wet sample FAD has a fluorescence peak at 535 nm with a FWHM of 85 nm and a 1.0 ns lifetime, where as the dry sample FAD has a fluorescence spectrum shift of 30 nm with the peak at 565 nm. Wet sample protoporphyrin IX exhibits two fluorescence peaks at 650 nm and 710 nm with FWHM of 35 nm and 50 nm respectively. Dry sample protoporphyrin IX exhibits also has two fluorescence peaks at the same wavelength as what was found on in wet state but has decreased peak intensity at 650 nm. The dry sample collagen type I has a broad fluorescence spectrum that peaks at 460 nm with a 0.45 ns lifetime. No fluorescence was measured from the wet collagen.
**Conclusion:** From these results, we conclude that there is a reasonable probability that further research will lead to rapid and non invasive method to identify melanoma.
This research was supported, in part, by a grant from the A.Ward Ford Foundation.

## 21

### MULTIMODAL CONFOCAL MICROSCOPY AS AN OPTICAL PATHOLOGY TOOL

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**Background and Significance:** Identification of malignant cells and small tumor nests *in vivo* and in real time is one of the major challenges in oncology. Therefore, there is a strong need for high-resolution detection and microscopically controlled removal of neoplasms, to ensure higher cure rate combined with maximum tissue preservation.
**Materials and Methods:** We have designed and implemented a multimodal multi-spectral confocal microscope that enables registration of reflectance, fluorescence, and fluorescence polarization images of pathological tissue. Illumination and acquisition of the microscope are optimized for the best performance in reflectance and fluorescence modes in the wavelength range between 600 nm and 1100 nm. The imaging device was tested using resolution targets, absorbing and fluorescent dye solutions with added scattering particles, and human tumor specimens. For quantitative assessment of fluorescence polarization the system was calibrated using the mixtures of aqueous and glycerol solutions of methylene blue or toluidine blue. Reflectance, fluorescence, and fluorescence polarization images of cancerous skin specimens stained using methylene blue or toluidine blue were obtained and compared to histology.
**Results and Conclusion:** The results of the study indicate that dye-enhanced confocal microscopy allows imaging of tissue structures with high contrast and resolution (lateral$\sim$1 μm; axial $\sim$5 μm) down to the depth of 200 μm. Confocal images of stained skin closely resemble corresponding H&E sections. Based on these results we concluded that the developed technique may provide an efficient real-time optical tool for diagnosing skin pathology.

## 22

### *IN-VIVO* DETERMINATION OF SKIN OPTICAL PROPERTIES USING DIFFUSE OPTICAL SPECTROSCOPY

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**Background and Objectives:** We have developed a modified two layer probe, which can be used in combination with diffuse optical spectroscopic (DOS) methods, to noninvasively determine full spectrum optical properties of superficial *in-vivo* skin in the wavelength range from 650–1000 nm. This new probe relies on a simple two-layer diffusion model to determine tissue absorption and reduced scattering coefficients. These properties can be translated into hemoglobin, melanin, and water concentrations.
**Study Design/Materials and Methods:** We have carried out steady-state frequency domain photon migration measurements using this new probe. By making the source-detector separation less than 3 mm and employing a Spectralon scattering layer, we are able to limit the interrogation depth to the top 1$\sim$2 mm of skin. We compare results obtained using this new probe with those acquired using a standard DOS probe and with the literature.
**Results:** We have determined full spectrum optical properties of *in-vivo* skin for three subjects including an African, an Asian, and a Caucasian. Furthermore, we were able to quantify melanin, hemoglobin and water concentrations from absorption spectra.
**Conclusions:** Optical properties obtained using this new probe demonstrate spectral features that are consistent with sampling of epidermis and dermis and are distinct from those obtained using the standard probe geometry, which interrogates a much larger volume of tissue.

## 23

### SCATTERING PROVIDES CONTRAST FOR THE DETECTION OF NONMELANOMA SKIN CANCER

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**Background and Objective:** Differences in absorption and/or scattering of cancerous and normal skin have the potential to provide a basis for non-invasive cancer detection. In this study we have compared the *in vitro* optical properties of human epidermis, dermis, and subcutaneous fat with those of nonmelanoma skin cancers, including infiltrative basal cell carcinoma, nodular basal cell carcinoma and squamous cell carcinoma, in the spectral range between 370 nm and 1600 nm.

**Materials and Methods:** Freshly discarded specimens of normal and cancerous human skin were obtained from surgeries under IRB approved protocol. The samples were briefly rinsed in saline solution and sectioned using microcryotome. The thickness of the tissue sections varied between 50 and 600 μm. Diffuse reflectance and total transmittance of the samples were measured using an integrating sphere spectrophotometer. Absorption and reduced scattering coefficients were calculated from the measured quantities using inverse Monte Carlo technique. The data were statistically analyzed using Student's t-test to evaluate the significance of the differences between optical properties of each normal tissue-cancer pair.

**Results and Conclusion:** The statistical data analysis revealed that there are significant differences ($p<0.05$) in scattering of cancerous and healthy tissues in the spectral range from 1050–1400 nm. The scattering of cancerous lesions is consistently lower than that of normal tissues in this spectral region. At the same time absorption of cancerous and normal tissue does not differ significantly, with the exception of nodular BCC, which exhibits significantly lower absorption as compared to normal skin. Therefore, the spectral range between 1050 nm and 1400 nm appears to be optimal for nonmelanoma skin cancer detection.

## 24

### MONITORING OF hALA INDUCED PDT IN RAT BLADDER CANCER BY OPTICAL SPECTROSCOPY

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**Background and Objectives:** Photodynamic therapy (PDT) has been shown to be effective for both malignant and non-malignant diseases. Monitoring of the tissue response to the treatment can provide important information to help optimize factors such as drug and light dose. Also, availability of oxygen during PDT is crucial for the photochemical reactions to function, so information about the oxygen saturation of the tissue is very useful.

**Materials and Methods:** 5-aminolevulinic acid hexylester (hALA) is a new photosensitizer which may improve PDT treatment. To test the efficacy of hALA induced PDT, a study on rats with superficial bladder cancer will be performed. A preliminary study has been carried out on four Fischer F344 female rats. After catheterization, a hALA solution was instilled in the bladder (1 h) and two hours prior to the photodynamic treatment. Different fluences below 25 J/cm$^2$ were tested. The illumination source was an Argon-pumped (Innova Coherent 70) dye-laser (Spectra Physics model 375) centered at 635 nm. A 200 μm fiber-optic applicator with an isotropic diffuser tip delivered the light to the rat bladder through an 18 gauge catheter inserted in the urethra.

**Results and Conclusions:** The results from this preliminary study is still under investigation, but seem promising. In the full scale study, in vivo reflectance and fluorescence spectroscopy will be carried out to monitor both the oxygen saturation and the potential changes in the rat bladder tissue before and after PDT treatment.

## 25

### INTRAOPERATIVE DISCRIMINATION OF GLIOMA CELLS USING QUANTUM DOTS

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**Background and Objectives:** Quantum dots (QDs) possess unique photo-physical properties that overcome some limitations of organic fluorophores and hold great potential for biological imaging applications. Use of QDs for *in vivo* biological applications is still in its infancy, however, due to the difficulty in modulating QD fluorescence to yield dynamic cellular information and cytotoxic effects of using CdSe QDs are still under debate as their integrity and stability over time is unknown. Currently, we are evaluating the feasibility of utilizing anti-EGFR conjugated QDs *in vivo* to identify malignant glioma cells, known to over-express EGFR, from surrounding healthy neural cells during surgery. This discriminatory power would facilitate increased accuracy of tumor cell resection, an ability that could impact survival times.

**Study Design/Material and Methods:** The LN229 glioma cell line was labeled with anti-EGFR conjugated 655 nm QDs and imaged *in vitro* using a fluorescent microscope, and *in vivo* in a nude mouse model using our Xenogen IVIS 200 system.

**Results:** Control experiments verify QD labeling specificity. Internalization of QDs was confirmed using confocal microscopy. Cell function and viability were evaluated *in vitro* following initial labeling by comparing mitochondrial function and apoptosis assays to control populations.

**Conclusion:** We will present an assessment of the correlation of QD labeling of implanted LN229 cells in nude mice with complete tumor resection as well as studying in greater detail the cytotoxic effects of QDs *in vivo*.

## 26

### SCATTERED LIGHT EXPOSURE OF THE EYE DURING PERIORBITAL LASER TREATMENTS

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**Background and Objective:** The treatment of facial tissue near the eye can potentially expose ocular tissues to scatted laser radiation that been diffusely scattered around optical tissues. This is of particular concern for the more penetrating laser wavelengths within the 600–1100-nm spectral region. The use of eye patches and occluders protect the globe from direct exposure; however, scattered energy from around the protector can reach the eye. The objective of this work was to explore a photographic method and a subjective method to estimate the fluence at different internal eye structures.
**Methods:** A charge-coupled-display (CCD) camera was used to measure the radiance (brightness) of scattered optical radiation in the orbital area when a low-power laser was placed at several periorbital locations. Subjective measures of brightness permitted determination of retinal exposure levels.
**Results:** The measured levels were scaled to 1 and 10-watt levels to ocular structures.
**Conclusion:** Significant levels of laser radiation can be transmitted to ocular tissues despite the use of lid covering, and great caution must be exercised in the choice of appropriate eye protection at wavelengths in the 600–1100 nm spectral region.

## 27

### ASSESSMENT OF OCULAR SAFETY OF MEDICAL AND COSMETIC PHOTOTREATMENT SYSTEMS WITH NON-UNIFORM LIGHT DISTRIBUTION

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**Background and Objective:** Recent developments in phototreatment technologies emphasize the need for rapid and reliable techniques to assess ocular safety levels of newly introduced laser- and light-based instruments. The difficulty of determining safety is confounded by increased sophistication and complexity of modern phototreatment devices, in particular, the advent of devices with intentionally non-uniform output distribution of optical energy. In addition, the safety levels often have to be assessed under two distinct sets of conditions: diffusely reflected light present during normal operation of the device and direct viewing of the output surface that constitutes an abnormal condition. The objective of this work was to address these needs and develop a procedure to determine accurately and expediently hazard levels for both laser and broadband systems.
**Methods:** The technique employs a calibrated CCD camera to measure the radiance (brightness) of either direct or scattered (diffusely reflected) optical radiation. For laser or incoherent sources, the radiance camera is calibrated with a radiometer using the same source as employed in the phototreatment application to properly account for the instrument's spectral and temporal response.
**Results:** The method was employed to perform safety assessment of a novel broadband IR device. The camera once calibrated was used to record the brightness profile of remitted light. The safety profile of the device was fully characterized for both viewing scenarios.
**Conclusion:** An effective means was developed to measure the potential retinal hazards of viewing either direct or remitted light from phototreatment devices using various sources of light, including those with non-uniform spatial distribution of optical energy.

## 28

### HISTOLOGICAL EVALUATION OF MINIMALLY INVASIVE RADIOFREQUENCY EXPOSURE

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**Background and Objectives:** Various non-invasive, non-ablative radiofrequency (RF) devices are available for the objective to provide skin tightening. However the achieved immediate and delayed skin tightening is only minimal, and collagen damage for clinically relevant exposure parameters could so far only be demonstrated by electron microscopy.
**Study Design/Materials and Methods:** Post mortem procured human skin samples were thermally equilibrated at 34°C and exposed to a prototype RF device that delivers the energy through a pair of fine needle electrodes. These needle electrodes have an electrically isolated shaft and a fine, non-isolated tip. The RF device has a maximal electrical output power of 100 W and provides pulses with preset pulse duration. The probe holder allows varying the distance between the needles. Various exposure parameters (power, pulse duration, needle distance and location) were tested. No skin cooling was employed. Thermal damage was assessed by NBTC staining of serial transverse and horizontal sections.
**Results:** We demonstrated individual, separated thermal lesions of elliptical shape with complete loss of NBTC staining and complete loss of collagen birefringence confined to the tip of the needles. The diameter of the lesions could be widely varied according to employed power and exposure time. For an exposure time of 2 seconds and a power of 70 W a diameter of 2 mm was measured. No evidence of epidermal thermal damage was detected. Marked immediate skin shrinkage was observed.
**Conclusions:** Minimally invasive RF exposure can provide subepidermal, confined thermal damage, sufficient to cause marked collagen denaturation and shrinkage, of virtually any diameter at any depth as determined by the location of the probe. It is feasible that this treatment concept offers distinct therapeutic advantages for skin tightening.

## 29

### ULTRASTRUCTURAL EVALUATION OF THREE NON-ABLATIVE DEVICES

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**Background and Objective:** There are several non-ablative rejuvenation devices that claim to deliver energy to the deep dermis in order to promote tissue contraction. This study will compare ultrastructural changes immediately after treatment with three non-ablative devices.
**Study Design/Materials and Methods:** One subject was consented and treated on the abdominal skin with the Syneron WR, Cutera Titan, and Thermage Thermacool devices using single and multiple pulses. Biopsies were taken immediately post treatment for electron microscopic examination. Samples from each 1 mm depth were examined by two blinded observers for ultrastructural changes.
**Results:** The tissue showed increased amount of collagen fibril changes with increasing number of passes. The changes seen after multiple passes showed an accumulation of partially denatured collagen and consolidation of inter fat septae. The Titan and Syneron were more superficial with single passes but with multiple passes the cooling was overcome showing some epidermal injury. The Titan and Thermacool demonstrate the most effect in the dermal fat interface with single and multiple passes.
**Conclusion:** This ultrastructural study shows changes in collagen fibril morphology with the greatest effect in the dermal fat interface with multiple passes in all devices. There are subtle differences between these devices. Clinical correlation is warranted.

## 30

### FLUORESCENCE DETECTION OF SMALL-CELL LUNG CANCER WITH A TARGETED SOMATOSTATIN AGENT

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**Background and Objectives:** Early, accurate detection of small-cell lung cancer (SCLC), before it becomes systemic, is essential for successful treatment. Fluorescence-based imaging provides safe, sensitive detection of malignancies. Targeted delivery of fluorophores increases sensitivity of endoscopic imaging.
**Study Design/Materials and Methods:** We synthesized novel somatostatin analogs, based on backbone-cyclic peptides, and conjugated them with fluorescent agents. Nineteen conjugates differing in core peptide, length of alkyl linker, and fluorescence moiety (rhodamine and fluorescein) were tested *in vitro*, using a standard radioligand receptor binding inhibition assay. Nine of the more promising conjugates were tested *in vivo* by fiber optic spectrofluorimetry and quantitative spectral imaging, on an H69 human SCLC tumor mouse xenograft model.
**Results:** The lead compound, a peptide (a cyclic analog of somatostatin 14), linked to fluorescein using γ-aminobutyric acid, showed exceptional tumor/normal tissue (T/NT) ratios: lung ($8.9 \pm 2.8$); skin ($9.6 \pm 3.1$); kidney ($27.2 \pm 3.4$); pancreas ($28.5 \pm 3.2$); liver ($48.2 \pm 5.2$) and spleen ($89.4 \pm 6.4$).
**Conclusions.** These T/NT ratios, ranging from 9 to 90, are an order of magnitude higher than reported for any other fluorophore conjugated with a somatostatin analog. They enable high-quality fluorescence imaging of SCLC overexpressing somatostatin receptors against a background of normal tissue and provide potential for improved endoscopic detection of early stage tumors, down to about 1 mm diameter. Our results demonstrate, for the first time, that human SCLC can be specifically targeted with high selectivity by a fluorescent bioconjugate of somatostatin analog, providing unmatched T/NT ratios.

## 31

### FLUORESCENCE LIFE TIME IMAGING FROM NEURONS AND SUBCELLULAR COMPONENTS DURING LOW INTENSITY LASER THERAPY USING FIBER-OPTIC NANO-PROBES

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**Background:** The objective of this paper is to design fiber-optic based nano-probes for precise delivery of laser light and for probing desired cellular organelles and study the mechanism of light interaction during low-level laser irradiations of tissues.
**Study Design/Materials and Methods:** Nano-probes are produced by drawing or etching the optical fiber tips to submicron size using a micro-puller or hydrofluoric acid bath. Then the fiber tip is coated with metal to minimize the leakage of light through fiber cladding. Various cell lines have been purchased from vendors and incubated. Laser and LED wavelengths are precisely delivered to different parts of the cell using a fiber nano-probe and the output fluorescence signals from target location is sensed by another fiber nano-probe which is connected to a fluorescent lifetime imaging microscope or photon counter for real-time measurements. Parametric studies have been performed with variation of laser power, wavelength and exposure time.
**Results:** Cells and cellular organelles are found to respond to light in a number of ways including rate of cell division, formation of singlet oxygen, and generation of ATP depending upon the light parameters and target location of laser beam.
**Conclusions:** Determination of the mechanism is important for optimization of therapies and for measurement of effectiveness of light doses.

## 32

### BLOOD DETECTION WITH MAGNETO-MOTIVE OPTICAL DOPPLER TOMOGRAPHY

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**Background and Objectives:** We introduce a novel contrast mechanism for imaging blood flow using magneto-motive optical Doppler tomography (MM-ODT).
**Study Design/Material Methods:** MM-ODT combines an externally applied temporally oscillating high-strength magnetic field with ODT to detect erythrocytes moving according to the field gradient. A solenoid cone-shaped ferrite core extensively increased magnetic field strength ($B_{rms} = 0.7$ Tesla, $\partial B/\partial z = 200$ Tesla/m) at the tip of the core and also focused the magnetic force on targeted samples.
**Results:** Hemoglobin contrast was demonstrated in a capillary tube filled with moving blood and a 10 day-old chicken embryo by imaging the Doppler frequency shift which was observed independent of blood flow rate and direction.
**Conclusions:** We have demonstrated what is believed to be the first implementation of MM-ODT for improved Doppler imaging of blood flow using an external oscillating magnetic field. The controlled and increased Doppler frequency in MM-ODT may provide a new investigational tool to study *in vivo* blood transport.

## 33

### MAGNETO-MOTIVE DETECTION OF SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES USING OPTICAL COHERENCE TOMOGRAPHY AND ULTRASOUND

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**Background and Objectives:** We investigate capability of optical coherence tomography (OCT) and ultrasound (US) to identify superparamagnetic iron oxide (SPIO) nanoparticles taken up by liver macrophages.
**Study Design/Material Methods:** We tested detection of nanoparticles by combining OCT and US with a high intensity time varying magnetic field (0.7T) applied to tissue containing SPIO nanoparticles. Six $ApoE^{-/-}$ high fat fed mice were sacrificed 9 days after intravenous injection of SPIO (1.5 mmol fe/kg). Livers were removed and imaged using OCT, differential phase OCT, and US. Livers from two control $ApoE^{-/-}$ mice were also imaged.
**Results:** Maximum tissue displacement of the nanoparticles observed by differential phase OCT was 200 nm. M-mode OCT scans of $ApoE^{-/-}$ mouse liver also demonstrated nanoparticle movement under focused magnetic field excitation. US Doppler showed a frequency shift in response to an applied magnetic field. In control livers, optical path length change, oscillation in M-mode scans, and frequency shift were not observed.
**Conclusions:** Results of our experiments indicate that OCT and US combined with magneto-motive excitation are candidate imaging modalities to identify macrophage cells containing SPIO nanoparticles.

## 34

### IMAGING TISSUE ENGINEERED VASCULAR GRAFTS WITH OPTICAL COHERENCE TOMOGRAPHY

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**Background and Objectives:** Optical coherence tomography (OCT) has been demonstrated to be an effective, minimally invasive tool to monitor the structural characteristics of tissue *in vivo* and *in vitro*. Several studies were performed to determine whether OCT would be a useful modality to evaluate the TEVG cellular layer during normal development and the layer's response to device implantation.
**Study Design / Materials and Methods:** We developed a side-firing OCT endoscope that obtains longitudinal scans within a sterile environment. The diameter of the endoscope is 2-mm and it has a resolution of approximately 15-um. Three sets of experiments were performed: a study of the robustness of the TEVG cellular layer to endoscopic imaging, a comparison between cell layer thicknesses measured from OCT images and histology, and a study of cellular layer response to a bare metallic stent within the TEVG.
**Results:** The endoscopic imaging was found to have no effect on the cellular layer while providing an accurate measure of cellular thickness. OCT was also capable of monitoring the proliferative response of the TEVG cellular layer due to the metallic stent implantation.
**Conclusions:** Optical coherence tomography can be an effective tool in the design of tissue engineered vascular grafts, and in the evaluation of vascular devices within a TEVG. These capabilities provide great promise for the assessment of vascular therapies.

## 35

### HYPOTHESIS TESTING TO DETECT BOUNDARIES IN MULTI-LAYERED ANISOTROPIC MEIDA USING POLARIZATION-SENSITIVE OPTICAL COHERENCE TOMOGRAPHY

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**Background and Objectives:** Polarization properties of tissues including form-birefringence, form-biattenuance and optic axis orientation are physiologically significant and correlate with the size, density and orientation of fibrous structures. Normalized Stokes vector trajectories measured by polarization-sensitive optical coherence tomography (PS-OCT) are employed to detect boundaries in multi-layered anisotropic tissue and determine form-birefringence, form-biattenuance and optic axis orientation.
**Study Design/Materials and Methods:** A hypothesis testing method is developed to detect automatically boundaries between layers by identifying discontinuities in the curvature of the normalized Stokes vector trajectory. Probability density functions of continuous curvature in each layer and discontinuous curvature between layers are determined by Monte Carlo simulation. The corresponding likelihood ratio is obtained and a threshold value is determined to test curvature values to identify boundaries.
**Results:** The hypothesis testing method was verified using simulated PS-OCT data with and without polarimetric speckle noise. The hypothesis testing method was also applied to PS-OCT data recorded from a porcine annulus fibrosis specimen to detect automatically boundaries and determine form-birefringence, form-biattenuance and optic axis orientation.
**Conclusions:** Hypothesis testing using the curvature of the normalized Stokes vector trajectory on the Poincaré sphere recorded by PS-OCT is an effective analytic-geometrical tool to detect boundaries in multi-layered anisotropic tissues.

## 36

### DETECTION OF PHOTOTHERMAL OPTICAL PATH LENGTH CHANGE IN TISSUE PHANTOMS BY DIFFERENTIAL PHASE OPTICAL COHERENCE TOMOGRAPHY

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**Background and Objectives:** Absorption of pulsed laser energy in a tissue generates photothermal (PT) effects such as refractive index change and thermoelastic expansion. Optical path length change in response to pulsed laser irradiation was measured in tissue phantoms by differential phase optical coherence tomography (DP-OCT). The effect of absorption and scattering on photothermal induced optical path length change was investigated.
**Study Design/Material Methods:** Dye (Eosin B) doped gelatin phantoms with different concentrations (0.2 mM, 1 mM, and 5 mM) were prepared and absorption ($\mu_a$) and scattering ($\mu_s$) coefficients were measured using a spectrophotometer. DP-OCT was applied to measure optical path length increase due to phantom heating following pulsed laser irradiation ($\lambda = 532$ nm and $\tau_p = 100$ ms).
**Results:** Measured optical path length increased monotonically with light absorption in the phantom. Higher dye concentration in phantoms resulted in a photothermal optical path length increase (2–20 nm) over one-order of magnitude. Measured optical properties showed that in comparison to scattering, absorption was dominant in phantoms with dye concentration less than 5 mM.
**Conclusions:** DP-OCT may be an effective tool to measure with high sensitivity depth resolved optical path length change in laser heated tissue phantoms with variable absorption and scattering.

## 37

### DISPERSION MEASUREMENT USING SPECTRAL DOMAIN OPTICAL COHERENCE REFLECTOMETRY FOR MATERIAL IDENTIFICATION

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**Background and Objectives:** Material dispersion results from spectral variation of refractive index. Although dispersion is problematic in certain biomedical imaging instrumentation such as optical coherence tomography (OCT) an alternative viewpoint is its measurement can be utilized to identify or characterize materials similar to conventional absorption and transmission spectroscopy.
**Study Design/Materials and Methods:** We report on a method to determine material dispersion from data recorded by a spectral domain optical coherence reflectometer that uses a broad-band frequency swept laser source. To accommodate non-uniform spectral sampling of the swept laser source, a non-uniform Fourier transformation (NUFT) is utilized to compute reflection magnitude in the time-delay domain. In addition, a multitaper spectral analysis method is applied to optimize statistical analysis of recorded spectral interference fringes from test samples.
**Result:** Comparison between dispersion of water determined from spectral domain optical coherence reflectometry data and absorption spectroscopy show excellent agreement in spectral variation.
**Conclusions:** Spectral domain optical coherence reflectometry in combination with advanced signal processing is a reliable method to measure material dispersion in weakly scattering test samples.

## 38

### OPTICAL COHERENCE TOMOGRAPHY AS AN AID IN CHEMOPREVENTION OF SKIN CANCER

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**Background and Objectives:** The current method of assessing chemopreventative agent efficacy in skin relies on biopsy. Optical Coherence Tomography (OCT) is a non-invasive imaging modality that can visualize skin morphology. Statistical analysis of OCT images may aid in determining efficacy of chemopreventive agents.

**Study Design/Materials and Methods:** The OCT system used in this study includes a superluminescent diode centered at 1310 nm with a 50 nm bandwidth. The axial and lateral resolutions were 16 and 15 μm, respectively. A $625 \times 800$ pixel image was acquired in 4 s. Each patient was imaged at 2 sun-protected locations and 2 sun-exposed locations, with a subsequent biopsy. Each location received a clinical diagnosis, a histological diagnosis, and a solar elastosis (SE) score. The images were statistically analyzed, and the results were compared to the histology.

**Results:** Statistically significant differences ($p < 0.02$) between image slope mean of $SE = 0$ and $SE = 2$ images was observed. We found trends as a function of age in image slope mean and mean pixel intensity. Visual differences were apparent in the OCT images of undiseased skin and actinic keratosis.

**Conclusion:** OCT may be used to evaluate the degree of sun damage in skin and identify pre-cancerous lesions.

## 39

### A COMPARISON OF MASS REMOVAL AND CRATER MORPHOLOGY PRODUCED IN CORTICAL BONE BY ABLATION USING SELECTED MID-INFRARED WAVELENGTHS OF A FREE ELECTRON LASER

**Jong-In Youn, George M. Peavy, and Vasan Venugopalan**

**Background and Objective:** A study of mass removal compared to crater morphology was investigated for cortical bone using selected mid-infrared laser wavelengths.

**Study Design/Materials and Methods:** The wavelengths examined were 2.79, 2.9, 6.1 and 6.45 μm produced by a Free Electron Laser (FEL) emitting 4 μs macropulses consisting of 1–2 ps duration micropulses delivered at 350 ps intervals. The mass removal measurement was conducted using a microbalance apparatus and crater morphology and collateral thermal injury produced were investigated by light microscopy following examination of histologic sections of specimens.

**Results:** The study demonstrated that the highest mass removal was achieved at $\lambda = 6.1$ μm followed by, in order, $\lambda = 2.9$, 6.45 and 2.79 μm. The zones of thermal injury and crater morphology at the selected wavelengths were examined at the radiant exposure of 28.3 J/cm$^2$. Ablation using $\lambda = 6.1$ μm provided the least collateral thermal injury with the largest crater size. The greatest amount of collateral thermal injury was produced by $\lambda = 2.79$ μm at sides and base of the ablation crater made in cortical bone.

**Conclusions:** The mass removal of cortical bone produced by FEL ablation at selected mid-IR wavelengths was measured as a function of incident radiant exposure. The ablation efficiency was found to be dependent upon wavelength and $\lambda = 6.1$ μm provided the greatest efficiency. The examination of collateral thermal injury and crater morphology confirmed that $\lambda = 6.1$ μm provides the least amount of collateral thermal injury with the largest craters.

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### ENHANCEMENT OF BOVINE BONE ABLATION ASSISTED BY A LIQUID LAYER CONFINEMENT

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**Background and Objective:** Several studies have shown that the application of a water layer to materials considerably augments ablation efficiency. The objective of this study was to characterize dominant mechanism(s) to determine ablation performances with and without a liquid layer.

**Study Design/Materials and Methods:** Q-switched Nd:YAG laser ($\lambda = 1064$ nm and $\tau_p = 30$ ns at FWHM) was used to generate optical breakdown and ablate bovine bone tissues. Distilled water was applied on the sample surface prior to each laser pulse. A photodetector was employed to monitor plasma formation, and acoustic measurements were conducted to evaluate pressure transients during ablation. Dynamics of bubble formation was appraised by pump-probe measurements to determine the effect of liquid vaporization. Volume of ablation craters was investigated by an Optical Coherence Tomography system, and ablation efficiency with and without a water layer was compared as function of radiant exposure and number of pulses.

**Results:** The addition of water resulted in lower damage threshold with 4 times higher acoustic transients and 6 times higher ablation volume. Bone ablation in air environment was contributed to shock wave impact during plasma formation whereas plasma confinement and bubble formation/collapse were thought to be responsible for ablation process in water. The ablation efficiency initially increased with radiant exposure but later became saturated due to plasma shielding effect.

**Conclusion:** Application of a liquid layer increased the ablation rate and lowers the damage threshold along with greater acoustic amplitude. However, plasma shielding limited acoustic pressure and ablation performance at higher irradiance.

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### HISTOLOGICAL QUANTIFICATION OF THERMALLY-MEDIATED COLLAGEN INJURY: A NOVEL AUTOMATED METHOD

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**Background:** Histological assessment of thermal injury usually relies upon qualitative bright-field examination even though quantitative polarized light methods have demonstrated efficacy. The latter approach has not been widely applied probably because of difficulties in making manual measurements. Recently, however, equipment has been developed to automate the process using a liquid-crystal compensator and circularly polarized light. We sought to validate this method.
**Methods:** Twenty-two Achilles tendon segments were placed in a water-bath (37–95°C) for 2 minutes. Five-micron sections, stained with picrosirius red, were analyzed to measure (1) collagen's optical retardation (OR); an indicator of molecular organization—thermal denaturation decreases molecular organization and hence reduces OR and (2) collagen fiber orientation; the degree of alignment was determined from the angular deviation (AD) of each distribution—the smaller the AD, the greater the alignment. Measurements ($n = 50$) were taken at 2 locations in each sample.
**Results:** OR was constant up to 60°C ($135 \pm 6$ nm), after which reduction occurred; $81 \pm 1$ nm at 65–70°C, $64 \pm 5$ nm at 75–80°C, and $55 \pm 3$ nm at 85–95°C; $P<0.05$. These changes were associated with tendon shrinkage and fiber straightening; AD decreased from 7.1° at 50°C to 4.8° at 70°C and 3.4° at 95°C ($P<0.01$).
**Conclusion:** Thermal injury to collagen is marked by progressive reduction in optical retardation and increased fiber alignment. Our data are consistent with previous qualitative and manually-measured quantitative results. Thus, the automated method permits rapid and accurate quantification of thermally-mediated structural changes in collagen fibers.

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### HEAT AFFECTED ZONE IN LAYERED TISSUE PHANTOM AND ANIMAL MODEL DURING SHORT PULSE LASER IRRADIATION OF TUMORS

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**Background and Objectives:** The objectives of this work were to analyze heat affected zones during short pulse irradiation of subsurface tumors by a novel approach of using a converging laser beam focused at tumor depth, and to compare results with those obtained using collimated laser beam.
**Study Design/Materials and Methods:** Using a mode-locked short pulse laser, experiments were performed on three-layered tissue phantoms containing inhomogeneities, *in vitro* samples of mouse skin tissue, and live anaesthetized mice with mammary tumors. Histological analyses of irradiated tissue samples were conducted to assess thermal effects on tissue architecture.
**Results:** For all the cases, desired temperature rise at the focal plane in conjunction with localized heat affected zone and minimal thermal spread were obtained using a focused beam. During irradiation of mammary tumors in mice, a steady state value of desired peak temperature rise is attained faster compared to the collimated beam case at the tumor location than the surrounding healthy tissues. Experimental measurements matched excellently with the prediction from non-Fourier heat conduction model as opposed to Fourier model.
**Conclusions:** Validation of measurements with numerical modeling results demonstrates that skin should be modeled as a multi-layered medium having layer-specific optical and thermo-physical properties rather than a single-layer medium. Histology of thin sections of plastic-embedded material provides high resolution images of heat-affected zones.

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### CONFINED THERMAL DAMAGE WITH INTENSE ULTRASOUND (IUS)

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**Background and Objective:** The objective of this study was to investigate the possibility of inducing a pattern of precise and predictable subepidermal thermal damage in human skin with Intense Ultrasound (IUS).
**Materials and Methods:** Experiments were performed with a newly developed IUS prototype device (Ulthera LLC, Mesa, AZ) on post-mortem human skin samples at 34°C in-vitro. For this study 7.5 MHz and 10 MHz transducers with nominal acoustic output powers of 25 to 45 Watts were used without active skin cooling. Exposure times ranged from 50 to 200 ms. Thermal damage patterns in the tissue were histologically assessed with a Nitro Blue Tetrazolium Chloride (NBTC) assay.
**Results:** Depth and extent of thermal damage could be arbitrarily controlled by proper selection of treatment exposure parameters (e.g. exposure time, focal depth, ultrasound frequency). It was possible to create individual lesions up to a depth of 5 mm within the dermis. Cone-shaped, subepidermal lesions of 1 mm cross section and a depth ranging from 1 to 5 mm were generated with the 7.5 MHz probe operating at 7.9 J. A pattern of individual lesions were achieved in the deeper dermis by applying the probe sequentially at different exposure locations.
**Conclusion:** A combination of appropriate exposure parameters and multiple exposure locations can create pre-determined thermal damage pattern within the dermis. IUS is a new and promising tool for the creation of precise volumetric thermal damage patterns with short exposure times without the need for active skin cooling.

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### VISCOELASTIC PROPERTIES OF SEPTAL CARTILAGE DURING THERMALLY INDUCED STRESS RELAXATION

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**Background and Objectives:** Laser cartilage reshaping (LCR) is a promising method of *in-situ* treatment of structural deformities in nasal septum, ears and trachea. Laser heating induces change in cartilage mechanical properties and produces relaxation of internal stress allowing formation of a new desirable shape. While some animal and preliminary human studies have demonstrated potential feasibility of LCR, clinical application of the method requires precise understanding of evolution of cartilage mechanical properties with temperature. The purpose of this study was to evaluate dynamic change in viscoelastic behavior of septal cartilage undergoing stress relaxation during laser heating.

**Study Design/Materials and Methods:** Cylindrical cartilage specimens harvested from porcine septum were subjected to step unconfined compression on mechanical testing stage. The specimens were heated uniformly with radio-frequency electric field (500 kHz) and axi-symmetrically with laser irradiation (Nd:YAG, 1.34 μm). Stress relaxation was monitored during and after heating procedure. In the uniform heating experiments storage and loss elastic moduli were determined as a function of thermal history and time from stress relaxation curves. The measured values of cartilage mechanical properties were used in a finite-element numerical model simulating stress relaxation in compressed cylindrical specimen to predict stress history during axi-symmetrical laser heating.

**Results:** Heating of cartilage results in temperature dependent acceleration of stress relaxation. Though both storage and loss equilibrium elastic moduli decrease with increase in peak temperature and heating time the most dramatic decrease in cartilage mechanical properties occurs at threshold temperature of $67 \pm 5$ deg C. The results of numerical simulation of laser heating demonstrate good correlation with experimentally obtained stress histories.

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### LIGHT SCATTERING SPECTROSCOPIC CHARACTERIZATION OF NORMAL AND HPV-TRANSFECTED EPITHELIAL CELLS

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**Background and Objective:** Human Papilloma Virus (HPV) infection is a significant risk factor for cervical, neck and head cancers. This study aims to determine the spectroscopic features generated by morphological changes such as the size and form factors of the cell nucleus, as they occur in neoplasia, by investigating the intrinsic light scattering properties of primary normal and HPV transfected human epithelial keratinocytes.

**Study Design/Materials and Methods:** Monolayers of human epithelial keratinocytes are investigated using Angular Light Scattering Spectroscopy (ALSS), a technique that acquires angular maps of the light backscattered by such cells over a large wavelength range (450–700 nm). Predictions from a Mie theory based model are then fit to the angular and wavelength dependence of the scattered light intensity. The results are compared with morphological studies of acridine orange stained cells with Confocal Fluorescence Microscopy ($\lambda_{exc} = 488$ nm; $\lambda_{em} = 520–560$ nm).

**Results:** ALSS discriminates between primary normal and HPV transfected human epithelial keratinocytes. Mie theory based fits of ALSS data and comparison with cell nuclear imaging studies suggest that the transverse diameter size of the nucleus influences strongly the ALSS signal.

**Conclusion:** The results indicate that ALSS can be used for the non-invasive monitoring of cell changes in neoplastic transformation associated with HPV infection.

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### AMPLIFIED LASER-NANOCLUSTER INTERACTION IN DNA, VIRUSES, BACTERIA, AND CANCER CELLS: POTENTIAL FOR NANODIAGNOSTICS AND NANOTHERAPY

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**Background and Objective:** The applications of nanotechnology in laser medicine are becoming an extensive area of research. The goal of this short review is to demonstrate a new strategy for photothermal (PT) nanodiagnosis and nanotherapy, based on integration of self-assembling absorbing exogenous nanoclusters into endogenous nanoclusters, followed by their pulse laser activation.

**Study Design/Materials and Methods:** The self-assembly of nanoparticles (gold nanospheres and nanorods, carbon nanotubes, etc.) in DNA, lipids, adenoviral vectors, *Staphylococcus aureus*, *Escherichia coli* and cancer cells were studied with PT, atomic force and electronic microscopy, depending on the nanovector and target type. The laser-nanocluster interactions were analyzed with a focus on PT phenomena, and their impact on living systems were verified with viability tests.

**Results:** Laser-induced optical, thermal, acoustic, and bubble-formation phenomena into nanoclusters may create linear and nonlinear and, especially, synergistic effects that are extremely sensitive to the spatial nanocluster reorganization during metabolism or therapeutic interventions. In particular, selective localized photodamage phenomena were observed in bacteria and cancer cells at relatively low pulse laser energy.

**Conclusion:** Non-linear amplification of laser-nanocluster interactions is a promising approach for both diagnosis and selective treatment of viruses, bacteria and cancer cells in various biological environments. Possible future applications include a combination of PT and gene cancer therapy.