IMAGING MULTIPLE MOLECULAR MARKERS UNDER SURGICAL ILLUMINATION

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Key Words: Image guided surgery, Near infrared imaging, molecular imaging.

Image-guided surgery (IGS) has proved to be an exceptional operational strategy for surgeons – aiding in performing far less invasive and much safer procedures. Among the optical imaging techniques available for IGS, near-infrared fluorescence (NIRF) imaging has been one of the main protagonists in research performed by both industrial and academic laboratories. The development of less toxic and far more specific molecular targeted probes constitutes a potential major improvement in the patient's surgery outcome by giving the surgeon the ability to make real-time clinically relevant decisions based on molecular labels. NIRF imaging has multiple advantages over visible fluorescence imaging, such as low tissue auto-fluorescence and low tissue scattering and absorption which has enabled capturing fluorescence signals several millimeters deep in the tissue. In addition, the excitation and emission spectra are invisible to the unaided human eye and so do not hamper the clinical workflow.

In this paper, we will describe a novel, high-resolution hexachromatic imager for NIRF imaging suitable for IGS. Our imaging sensor combines an array of vertically stacked CMOS photodetectors with pixelated spectral interference filters. The three-dimensional photodetector array exploits the silicon wavelength-dependent depth absorption coefficient to resolve trichromatic information at each pixel location. The pixelated interference filters following a chessboard layout act as shortpass and longpass filters on half of the pixels, respectively. High optical density is achieved due to a specialized nano-fabrication process where material layers with high and low dielectric constants are stacked. The overall result is a monolithically integrated hexachromatic imager capable of color reconstruction in half of the pixels and NIR-shade sensitivity in the other half of the pixels for multiple NIR fluorophore imaging for IGS.

The image sensor is used in conjunction with two NIRF tumor targeted probes to image murine prostate cancer. We demonstrate that by combining information from both probes, the sensitivity and specificity for detecting cancerous tissue is improved. The imaging system was also translated in the operating room to simultaneously image indocyanine green and methylene blue markers for sentinel lymph node mapping in patients with breast cancer. Data from this clinical study will be presented.

MAKING A DIFFERENCE IN SURGICAL ONCOLOGY – IDENTIFYING THE SENTINEL MARGIN

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Key Words: Therapeutic antibody, molecular imaging, intraoperative, oncology surgery, surgical margins. Epidermal growth factor receptor (EGFR)

Purpose
Despite major advancements in surgical oncology, the positive margin rate for primary head and neck cancer resection remains around 15-30%. Inadequate margins are directly correlated to poor survival, and as such, mitigation of these rates is critical to improve patient outcomes. We have developed an *ex vivo* imaging strategy that utilizes fluorescence intensity-peaks (relative to background signal) to locate potential close or positive margins on the deep surface of the resected tumor specimen.

Experimental Design
A clinical trial with over 50 patients with head and neck cancer scheduled for surgery received systemic administration of a tumor-specific contrast agent (panitumumab-IRDye800). After surgical resection, the tumor specimen was imaged in vivo during surgical resection and using a 3D specimen mapping device with optical capability. The three highest fluorescence intensity-peaks on the deep surface of the specimen were isolated and correlated to histology to determine the margin distance at these regions.

Results
Relative fluorescence peak-intensities identified the closest margin on the deep surface of the specimen within 2.5 minutes. In vivo imaging identified multiple areas that would be considered management changing events. The highest intensity-peak consistently (100%) detected the closest margin to the tumor.

Conclusion
Fluorescence intensity-peaks can identify the region on the specimen where tumor is closest to specimen's edge on the deep surface. This technique could have broad applications in obtaining adequate margins in oncological surgery.

ADVANCES IN SCAPE MICROSCOPY FOR HIGH-SPEED VOLUMETRIC IMAGING

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DEVELOPMENT OF COMPACT ULTRAFAST FIBER LASER SOURCES FOR NONLINEAR OPTICAL MICROSCOPY

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OCT FOR IMAGE-GUIDED THERAPY AND SURGERY

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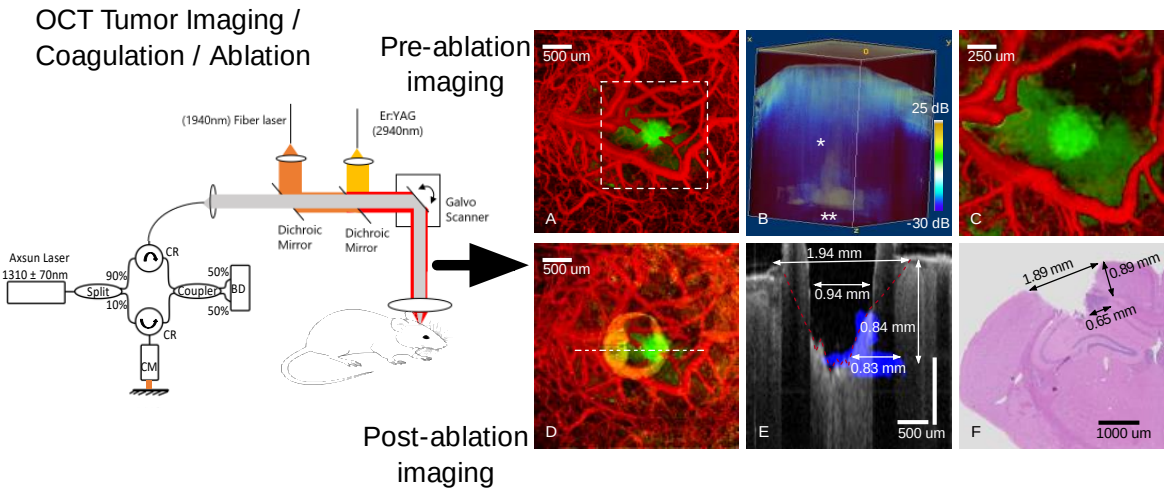
Key Words: laser, imaging, surgery, optical coherence, tomography, cancer

Image-guided therapy is a central enabling element for realization of precision medicine in the twenty-first century. Optical coherence tomography (OCT) can provide high-speed tomographic images of tissue with satisfactory contrast to differentiate blood flow, anomalous scattering constituents (e.g., tumors) and birefringent structures. In some cases, OCT can be integrated into an existing surgical workflow and benefit all stages of therapy including planning, real-time feedback control and post-treatment assessment. Today OCT is an integral component in some existing cardiovascular and ophthalmologic surgical workflows. An OCT-guided laser surgical system is presented for excision of brain tumors in an in vivo murine xenograft model. Precise and bloodless tumor resection under OCT image guidance is demonstrated so that tumor margins and vasculature are detected without exogenous contrast agents. The novel imaging capabilities and contrast provided by OCT can be utilized for more fundamental studies to investigate laser-tissue interactions in real-time. Two important laser-tissue interactions investigated with OCT are presented: laser coagulation of blood vessels and ablation of tissues. Study results demonstrate that OCT imaging of photocoagulation can enable a new understanding of important mechanisms relevant to laser photo-thermolysis of blood vessels. Similarly, OCT studies of the underlying mechanisms in laser tissue ablation motivate a novel approach for efficient and high-aspect ratio cutting for surgery.



RADIATION-NANO-OPTICAL INTERACTIONS

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The convergence of photonics with nanotechnology and/or ionizing radiation offers new capabilities for biomedical applications. Thus, in the *photonics-nano* convergence, nanoparticles can be used for a range of purposes: to enhance or spatially localize light-based treatments such as in phototherapy or photodynamic therapy; for targeted delivery of photoactive drugs such as photodynamic sensitizers; to provide new sources or amplify existing sources of contrast in cell and tissue imaging and to enable multiplexed optical biosensing; for light release of therapeutic agents such as drugs or genes; and as photosensitizers *per se*. Nanoparticles can also be used to enhance applications in the *nano-radiation* convergence, for example through increasing the effective radiation dose or increasing the oxygenation of tissue to overcome tumor hypoxia that limits tumor cell kill. In the *radiation-photonics* convergence, X-rays or radionuclides can be used to overcome the limited penetration of light in tissue that often hinders biophotonics applications, either by direct or Cherenkov-light mediated molecular excitation, thereby extending the capability of phototherapeutics and photodiagnostics. Finally, in the 3-way *photonics-radiation-nano* convergence, nanoparticles can further amplify ionizing radiation-optical interactions for treatment or imaging.

THE CURRENT STATE OF FLUORESCENCE GUIDED SURGERY

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Key Words: molecular, fluorescence, robotics, surgery,

Molecular guided surgery has made significant progress in the past decade, partially evidenced by the number of articles published and presented at scientific congresses. The field has had some successes and some failures, but excitement (and funding opportunities!) remain.

While the bulk of the more than 130 imaging agents historically used in the US were approved in the 1980's and 1990's, there has been renewed interest in fluorescent agents from the mid-2000's onwards. While the reasons for this could stem from the widespread commercialization of magnetic resonance and positron emission tomographic machines in the 1980's, recent years have witnessed the proliferation of clinically available fluorescent imaging systems – open, laparoscopic, robotic and microscope-based. Unfortunately, this has coincided with a consolidation of private entities performing medical imaging agent research & development, however new mechanisms of government funding have provided reasons for enthusiasm.

This talk will cover recent approvals, clinical studies and issues encountered therein.

TUNING ENGINEERED NANOMATERIALS FOR CANCER TREATMENT WITH REACTIVE OXYGEN SPECIES

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Key Words: Radiation, cancer, nanomaterials.

Engineered nanomaterials that produce reactive oxygen species while exposed to X- and gamma rays offer promise of a novel cancer treatment strategy. Similar to photodynamic therapy (PDT) but suitable for deep tumours, the new approach called X-PDT is highly effective at clinically low radiation doses. The X-PDT nanomaterials can enhance cancer radiotherapy, by increasing its selectivity, and decreasing side effects. Additionally, the nanomaterial platform offers therapeutically valuable functionalities such as molecular targeting, the capability for drug/gene delivery, adaptive responses allowing triggering of drug release and more. The potential of such nanomaterials to be combined with radiotherapy has been widely recognised, as apparent in an explosion of new advances in X-PDT. So far, the field seems to develop organically, by a combinatorial approach, and optimally designed materials and quantitative approaches remain scarce. In order for further breakthroughs to be made, and to facilitate clinical translation the applicable principles and fundamentals should be articulated. We will introduce mechanisms and principles underpinning rational material design for X-PDT. The understanding of these principles will enable novel ways to optimise the ROS yields and the ensuing cytotoxicity which is directly related to therapy success. The X-PDT nanomaterials will be discussed though the lens of catalytical processes at solid surfaces. Drawing on analogies between photo- and radio-catalysis, we propose that future authors build on selected advances in the areas of clean energy, water splitting and environmental remediation.

Traditionally, in PDT photosensitizer drugs act as molecular catalysts. In the X-PDT approach, catalysis takes place on the solid surface of nanomaterials. Many aspects of such surfaces are well established in solid state physics, but disciplinary barriers prevent wider utilisation of that knowledge to build optimised X-PDT agents. We discuss optimising of charge transfer catalysis where ROS are formed by redox reactions. The alternative process of energy transfer catalysis is also highly relevant, while much less understood; we discuss resonant tuning of X-PDT offered by energy transfer processes which offers unprecendented amplification.

Functionalisations and coatings are a ubiquitous feature of engineered nanomaterials and these layers can be used for facile tuning of X-PDT. We discuss how ionic organisation of fluid at the solid-liquid interface affects the potential profile, and how this, in turn, allows to easily adjust the matching of electron and hole energies with relevant redox potentials.

We then focus on nanomaterials where coatings contain clinical photosensitizers (a development analogous to surface-immobilised organocatalysts in chemistry). These offer additional dimension to optimise X-PDT, as they can utilise energy transduction (X-rays into visible light) and also Cherenkov radiation produced during radiotherapy. Furthermore we explore clinical translation of these materials where we discuss biocompatible nanocarriers (liposomes and PLGA nanoparticles) as well as mesoporous silica etc. Critically, the photosensitizers respond with ROS not only to light but also to radiation. We explain the underpinning mechanism which makes it possible to create X-PDT nanoparticles with sophisticated functionalities such as X-ray triggering exclusively from FDA-approved components, a step that brings closer their clinical translation. Finally in this section we draw attention of the reader to novel photosensitizers derived from aggregation-induced-emission (AIE) molecular species. We explain how their violation of the Kasha rule enables exceptionally high ROS yields, suggesting that they may help build uniquely powerful and clinically compatible nanoconstructs for X-PDT.

We then turn attention to cells and explore ways in which cells fight back the ROS attacks which is necessary for their survival. Effective X-PDT agents should be able to weaken or, ideally, disable this cellular protection. We focus on DNA damage and its repair, as well as on maintaining the redox status through the cellular antioxidant system based on glutathione system. Both DNA damage and the antioxidants can be interfered with using functional nanoparticles.

As a conclusion we present a roadmap for designing nanomaterials with optimised X-PDT performance.

SHEDDING LIGHT ON THE EFFECTS OF RADIATION THERAPY ON CIRCULATING TUMOR CELLS

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Key Words: Diffuse, fluorescence, sensing, circulating tumor cells, radiation therapy

Many common treatments for cancer – including radiation therapy (RT) – have the unfortunate side effect of promoting the spread of cancer to other organs [1-3]. While the 'pro-metastatic' effects of RT have been known for some time, it has garnered renewed attention in recent years in part due to the widespread study of circulating tumor cells (CTCs). In hematogenous metastasis, CTCs detach from the primary tumor and spread via the blood to other organs and tissues of the body. There are three main hypotheses for RT induced metastasis (RTIM) as reviewed in [1]: i) RT causes disruption of the primary tumor and vasculature, which leads to immediate shedding of CTCs, iii) RT induces biomolecular changes in tumor cells, such as epithelial to mesenchymal transition, leading to increased CTC shedding over time as the tumor cells die, and, iii) Systemic effects, such as the elimination of suppressive signaling molecules by the primary tumor resulting in the proliferation of existent but previously dormant micro-metastases [3].

Our team recently developed a new instrument called 'Diffuse *in vivo* Flow Cytometry' (DiFC; *figure 1*) [4]. The main advantage of DiFC is that it samples large circulating blood volumes (hundreds of µL per minute), allowing in vivo detection of very rare CTCs. DiFC uses specially designed fiber-optic probe bundles with built-in filters and lenses for efficient collection of weak fluorescent signals and blocking of tissue autofluorescence. As labeled cells pass through the DiFC field of view, transient fluorescent peaks are detected. A custom signal processing algorithm allowed us to determine the number, direction, speed, and depth of circulating cells, and reject false alarm signals from motion artifacts. For example, we recently showed that DiFC allowed detection of early dissemination of green fluorescent protein (GFP)-labeled multiple myeloma cells in a disseminated xenograft model at CTC burdens below 1 cell per mL, as well as rare CTC clusters (*fig. 1*).

In this presentation, we first discuss the design and prior validation of the DiFC instrument. Second, we discuss our recent work in application of DiFC to the study of RTIM. Specifically, we grew sub-cutaneous Lewis Lung Carcinoma (LLC) tumors in mice, which are known to aggressively metastasize to the lungs via the vasculature. Irradiation of the primary LLC tumors is known to significantly increase pulmonary metastases in this model, by as much as a factor of 5 [3]. We performed local RT of LLC tumors with a Small Animal Radiation Research Platform (SARRP). We measured CTC and CTC cluster numbers with DIFC in response to single or fractionated RT doses, compared to un-irradiated controls. We also monitored metastases in the lungs by BLI imaging, weight, and histology. DiFC revealed that LLC-CTC numbers significantly increased during tumor response in RT mice versus controls, and that these correlated with lung tumor burden at sacrifice. We also discuss future prospects for the use of DiFC in monitoring CTC response to other therapies.

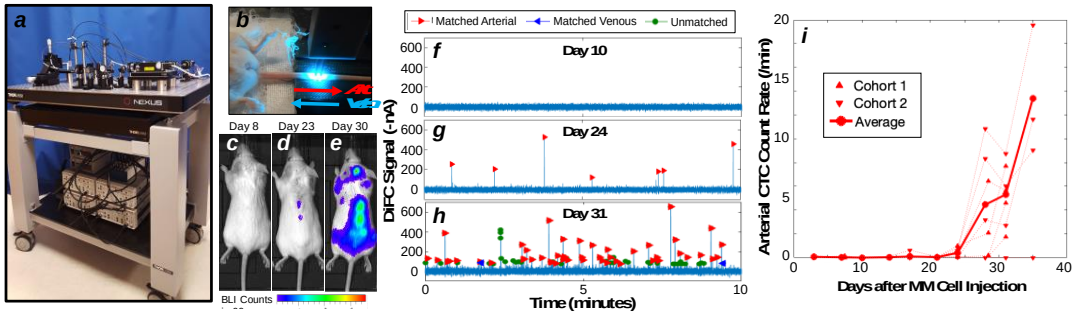

Figure 1. (a) DiFC instrument [4,5] designed to detect fluorescently-labeled CTCs in (b) mouse tail vasculature. We used DiFC to study growth of multiple myeloma in a mouse model, which was also monitored by BLI (c-e). Increasing numbers of CTCs were detected (f-h) in arterial blood (red arrows) as the (i) disease progressed.

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INCREASING DIFFUSE CORRELATION SPECTROSCOPY DEPTH SENSITIVITY AND BRAIN BLOOD FLOW SPECIFICITY

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Key Words: Near-infrared spectroscopy, diffuse correlation spectroscopy, acousto-optic modulation, cerebral blood flow, neuro-monitoring.

Continuous, accurate monitoring of cerebral perfusion may reduce morbidity and mortality in patients in critical care. While physiological monitoring helps assess the impacts of perfusion changes in the brain, a technology able to directly, continuously and non-invasively monitor cerebral blood flow (CBF) is needed.
Diffuse correlation spectroscopy (DCS) is an established optical modality which enables non-invasive measurements of cerebral blood flow (CBF). Similar to near-infrared spectroscopy (NIRS), DCS uses red and near-infrared light to interrogate biological tissue, but, instead of quantifying hemoglobin concentration and oxygenation from measures of light attenuation, DCS quantifies an index of blood flow (BF_i) by measuring the light intensity temporal fluctuations generated by the dynamic scattering of moving red blood cells. The technology has been extensively validated against gold standards and its clinical utility in infants has been demonstrated. As with continuous-wave (CW) NIRS, the effectiveness of CW-DCS in measuring CBF is hampered in the adult population by limited depth penetration and extra-cerebral contamination. These two limitations so far have precluded the wide adoption of optical monitoring techniques in health care settings.
Our goal is to advance DCS to see deeper into the human brain and to isolate cerebral blood flow from scalp signals. To this aim we have developed a range of approaches which, either individually or together, significantly improve performance over current CW-DCS technology.

We have first proposed and demonstrated operation of DCS in the time-domain (TD-DCS)[1], which offers dramatic improvements in brain sensitivity when considering late arriving photons. Transform limited, high power, Gaussian pulse-shaped laser sources and photon counting detectors with high efficiency and temporal resolution, and reduced after-pulsing probability, dark count rate, jitter and hold-off time are needed to maximize performance. Our current prototype uses commercially available components with several limitations[2], which we are trying to overcome by developing custom components. For the detectors, a commercially available solution is the use of superconducting nanowire single-photon detectors (SnSPD). These detectors reach up to 90% efficiency, have extremely low noise and great timing response, but they are very expensive and require a bulky cryogenic cooling system with a turn-on time of several hours.

The use of SnSPDs allows us to use longer wavelengths in the range of 1064 nm, instead of the typical NIRS wavelengths (680-850 nm). At 1064 nm absorption is comparable, but the scattering is considerably lower than at 800 nm, providing increased penetration depth. The use of longer wavelengths also offer a 15-20 times increase in the number of photons available for detection.
Another novel technology we are developing in our lab is acousto-optic modulated interferometric DCS. Instead of resolving in time photons that have travelled longer paths, we use short pulse ultrasound plane waves to tag the light field at specific depths, permitting separation of cerebral from extra-cerebral blood flow. This method in combination with a heterodyne interferometric detection technique permits separation of the light that was frequency shifted by the ultrasound. Moreover, this method allows us to use camera based parallel multi-speckle detection instead of photon counting detectors which are less costly and are commonly available with sensitivity and appropriate speed in the >1000 nm spectral range.

These emerging technologies are developed with the contribution of my coworkers at the Optics @ Martinos, and of our collaborators at Boston University and MIT Lincoln Laboratory.
This research is supported by NIH R01EB025145. The author hold patents on this technology.

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WHAT DOES LASER SPECKLE CONTRAST IMAGING REALLY MEASURE?

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NON-INVASIVE OPTICAL MEASURMENT OF CEREBRAL CRITICAL CLOSING PRESSURE IN PEDIATRIC HYDROCEPHALUS

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Key Words: Diffuse correlation spectroscopy; hydrocephalus; critical closing pressure; intracranial pressure

Hydrocephalus is a common disorder of cerebral spinal fluid (CSF) physiology that results in elevated intracranial pressure (ICP) and progressive expansion of cerebral ventricles.[1] It affects 1-2 of every 1000 live births, making it the most common disease treated by pediatric neurosurgeons in the US.[1] In roughly half of infants with hydrocephalus, ventricular expansion requires surgical intervention whereby a shunt is placed in the ventricles to divert CSF and relieve elevated ICP. Although timely treatment of elevated ICP is important for brain tissue viability, its implementation is hindered by the lack of tools for non-invasive ICP measurement. This study aims to validate non-invasive intracranial pressure (ICP) assessment with the near-infrared diffuse correlation spectroscopy (DCS) technique in infants with hydrocephalus.

DCS employs near-infrared light to measure local, microvascular cerebral blood flow (CBF) continuously at the bedside. In addition to CBF, a novel approach for measurement of cerebral critical closing pressure (CrCP) based on DCS measurements of pulsatile CBF in arterioles was recently demonstrated.[2-4] CrCP, which depends on ICP, defines the arterial blood pressure at which CBF approaches zero. Intraoperative non-invasive CrCP measurements with DCS on the prefrontal cortex were performed concurrently with invasive ICP measurements in 9 infants with hydrocephalus at the Children's Hospital of Philadelphia. Invasive ICP was measured during surgical shunt placement.

A significant correlation (R^2=0.6) between non-invasive CrCP and invasive ICP measures was observed (Figure 1). CrCP overestimated ICP at lower ICP levels, but was close to ICP at higher ICP levels.

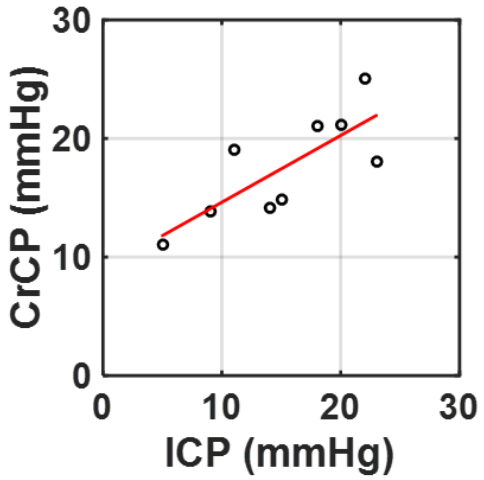

Figure 2 – CrCP measured by DCS (vertical axis) plotted against invasively measured ICP (horizontal axis) in 9 infant hydrocephalus patients. Solid red line is the linear best-fit (R^2=0.6, slope (95CI) = 0.6 (0.2, 1.0)).

This pilot data shows the potential of using DCS measurement of CrCP for non-invasive detection of elevated ICP in children. DCS probes are further well-suited for continuous, long-term monitoring. We hypothesize that CrCP overestimation of ICP at lower levels is because of CrCP's sensitivity to vasomotor tone. Enrollment of more patients is underway.

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BIOMECHANICS OF CELLS AND TISSUES: WHAT CAN WE LEARN WHEN WE COMBINE MECHANICAL STIMULI WITH MICROSCOPY?

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Key Words: Indentation, Tissue Mechanics, Brain, Embryology, Skin

Understanding the mechanical properties of biological tissues can shed light on how those tissues work and why, at times, they lose their functionality. Furthermore, a full characterization of a tissue's viscoelastic behavior may provide relevant hints for tissue reparation and tissue engineering. To measure these properties in in-vitro or ex-vivo experiments, researchers often make use of indentation instruments, which looks at how a material deforms under the effect of a calibrated mechanical load. In the first part of my talk, I will show how this technique can be used to determine the mechanical properties of brain slices, and I will comment on which kind of information those measurements can provide. I will show, for instance, that different regions of the brain have remarkably different viscoelastic properties, which seem to be correlated with the cell density measured, in a parallel experiment, via fluorescent microscopy.

As this example highlights, indentation measurements alone are often not sufficient to understand why certain tissues have certain mechanical properties. Under a (not transparent) surface, biological materials are often inhomogeneous and anisotropic. Because the indentation stress propagates several microns deep into the sample, without a proper imaging tool coupled to the indentation instrument, it is impossible to extract useful information on the mechanics of the material the sample is made of. As a point in case, I will show our latest measurements of the mechanical properties of chick embryos, where, combining indentation with optical coherence tomography (OCT), we could precisely map the stiffness of the spine from head to tail – a measurement that may provide interesting cues in the analysis of somites formation and growth. I will also show how the combination of indentation and OCT might find its way in scar and burn classification, introducing a new instrument for skin characterization that our group has just recently completed. Finally, I will show some preliminary results on the use of multiphoton imaging for tissue mechanics characterization. In this last part of the talk, I will show that it is indeed possible to look at the displacement and deformation of cells in a thin slice of tissue while the tissue is compressed by a calibrated mechanical stroke. This approach may pave the way for a much more thorough analysis of the origin of certain mechanical properties of tissues, where the contribution of the individual cells to the viscoelastic features of the materials can be finally disentangle from that of the extracellular matrix.

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Declaration of interest: Davide Iannuzzi is founder, shareholder, and advisor of Optics11.

TRANSLATIONAL SHEAR-WAVE OPTICAL COHERENCE ELASTOGRAPHY

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Key Words: Optical Coherence Elastography; biomechanics; eye; systemic sclerosis

The biomechanical properties of tissues can be dramatically altered by various diseases, such as keratoconus for the cornea of the eye and systemic sclerosis for the skin. Therefore, the ability to measure tissue biomechanical properties could provide critical information for assessing its health and detecting disease etiology as well as monitoring disease progression. Here, we present pilot results in development of noncontact dynamic optical coherence elastography (OCE) technique to evaluate the biomechanical properties of the cornea and skin of healthy subjects and those affected by diseases.

For systemic sclerosis (SS) disease, we demonstrated the first use of a combined OCT and OCE analysis for assessment of SS severity in 8 patients and 10 healthy subjects. Comparison to clinical diagnoses including skin biopsy, modified Rodnan skin score (mRSS) and site specific MRSS (SMRSS) was performed to validate the OCE technique. The OCE imaging was performed on the forearm after the 3D OCT scan to measure dermal thickness (DT). For SSc detection, the scattering coefficient was calculated based on the OCT signal slope (OCTSS) and the Young's modulus of the skin was quantified using the surface wave equation. The OCT and OCE results clearly differentiate the healthy and SSc patients and show strong correlations with the standard clinical evaluation techniques (Fig 1). These results show promise for clinical applications of OCT combined OCE for detecting diseases such as SSc.

Pilot experiments were also performed to assess biomechanical properties of cornea of 19 eyes in 12 human subjects. Dynamic submicron corneal surface wave deformation responses were measured with excellent repeatability over a wide amplitude range. Measured surface wave velocity ranged from 2.2 to 6.6 m/s between participants and correlated highly with intraocular pressure (r2 =0.58), but not central corneal thickness (r2 =0.018). These results demonstrate the first use of OCE to characterize biomechanics of human cornea in vivo with potential clinical applications for quantitative determinations of corneal biomechanics and IOP.

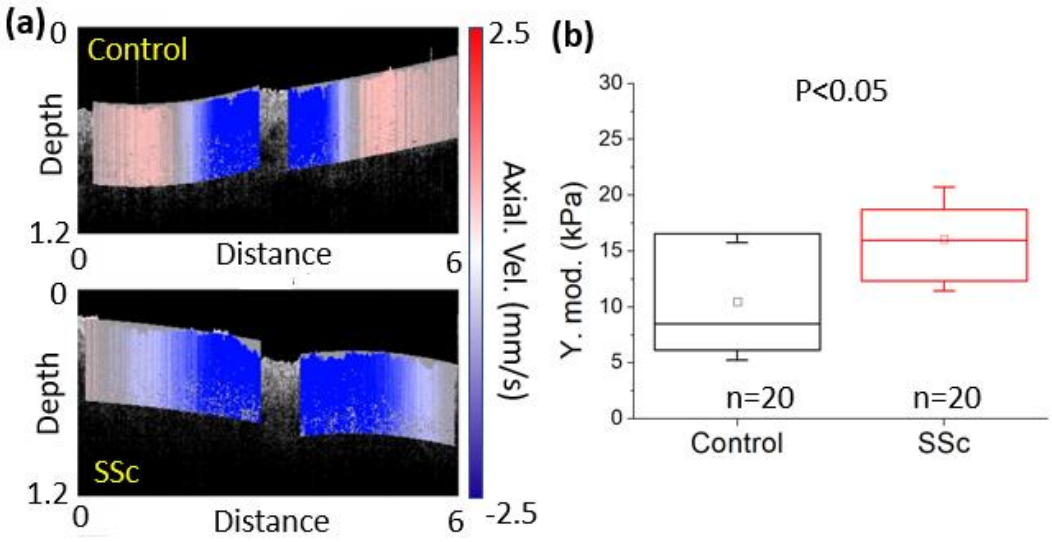

Figure 3 – Results of OCE measurements on the forearm of healthy and SS subjects. (a) OCT images showing surface wave propagation and (b) the Young's modulus between the control and SSc groups.

DIAGNOSIS OF INTESTINAL FIBROSIS BY SPECTROSCOPIC AND STRAIN PHOTOACOUSTIC IMAGING

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THE CURRENT STATE OF FLUORESCENCE GUIDED SURGERY

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Key Words: molecular, fluorescence, robotics, surgery,

Molecular guided surgery has made significant progress in the past decade, partially evidenced by the number of articles published and presented at scientific congresses. The field has had some successes and some failures, but excitement (and funding opportunities!) remain.

While the bulk of the more than 130 imaging agents historically used in the US were approved in the 1980's and 1990's, there has been renewed interest in fluorescent agents from the mid-2000's onwards. While the reasons for this could stem from the widespread commercialization of magnetic resonance and positron emission tomographic machines in the 1980's, recent years have witnessed the proliferation of clinically available fluorescent imaging systems – open, laparoscopic, robotic and microscope-based. Unfortunately, this has coincided with a consolidation of private entities performing medical imaging agent research & development, however new mechanisms of government funding have provided reasons for enthusiasm.

This talk will cover recent approvals, clinical studies and issues encountered therein.

ARTIFICIAL INTELLIGENCE (AI)-ENABLED MULTIMODAL MACROSCOPIC OPTICAL SPECTROSCOPY IMAGING PLATFORM FOR SURGICAL ONCOLOGY APPLICATIONS

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MAKING SENSE IN SURGERY USING NEAR-INFRARED OPTICAL IMAGING

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Key Words: Image-Guided Surgery, Fluorescence Imaging

There is a pressing clinical need to provide image guidance during surgery. Currently, assessment of tissue that needs to be resected or avoided is performed subjectively leading to a large number of failures, patient morbidity and increased healthcare cost. Because near-infrared (NIR) light propagates deeply within living tissues and interacts with molecular constituents, it offers unparalleled capabilities for objectively identifying healthy and diseased tissue intraoperatively. These capabilities are well illustrated through the ongoing clinical translation of fluorescence imaging during oncologic surgery. In this presentation, we will review our efforts to provide real-time & wide-field image-guidance during surgery using NIR diffuse optical imaging. We will present our latest results in fluorescence and endogenous imaging towards real-time monitoring and image-guided surgical intervention.

SCALABLE AND RELIABLE DEEP LEARNING FOR COMPUTATIONAL MICROSCOPY IN COMPLEX MEDIA

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Key Words: Deep learning, uncertainty quantification, speckle correlation, computational microscopy.

Emerging deep learning based computational microscopy techniques promise novel imaging capabilities beyond traditional techniques. In this talk, I will discuss two microscopy applications.

First, high space-bandwidth product microscopy typically requires a large number of measurements. I will present a novel physics-assisted deep learning (DL) framework for large space-bandwidth product (SBP) phase imaging [1], enabling significant reduction of the required measurements, opening up real-time applications. In this technique, we design asymmetric coded illumination patterns to encode high-resolution phase information across a wide field-of-view. We then develop a matching DL algorithm to provide large-SBP phase estimation. We demonstrate this technique on both static and dynamic biological samples, and show that it can reliably achieve 5× resolution enhancement across 4× FOVs using only five multiplexed measurements. In addition, we develop an uncertainty learning framework to provide predictive assessment to the reliability of the DL prediction. We show that the predicted uncertainty maps can be used as a surrogate to the true error. We validate the robustness of our technique by analyzing the model uncertainty. We quantify the effect of noise, model errors, incomplete training data, and “out-of-distribution” testing data by assessing the data uncertainty. We further demonstrate that the predicted credibility maps allow identifying spatially and temporally rare biological events. Our technique enables scalable DL-augmented large-SBP phase imaging with reliable predictions and uncertainty quantifications.

Second, I will turn to the pervasive problem of imaging in scattering media. I will discuss a new deep learning-based technique that is highly generalizable and resilient to statistical variations of the scattering media [2]. We develop a statistical ‘one-to-all’ deep learning technique that encapsulates a wide range of statistical variations for the model to be resilient to speckle decorrelations. Specifically, we develop a convolutional neural network (CNN) that is able to learn the statistical information contained in the speckle intensity patterns captured on a set of diffusers having the same macroscopic parameter. We then show that the trained CNN is able to generalize and make high-quality object predictions through an entirely different set of diffusers of the same class. Our work paves the way to a highly scalable deep learning approach for imaging through scattering media.

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PHOTOACOUSTIC MICROENDOSCOPY THROUGH MULTIMODE FIBERS

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DEVELOPMENT OF THE MEDIBEACON TRANSDERMAL GFR MEASUREMENT SYSTEM

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Key Words: transdermal fluorescence, fluorescence tracer agent, kidney function, glomerular filtration rate, medical device

Current methods of kidney function monitoring, based on plasma creatinine concentration, suffer from poor accuracy, lack of sensitivity, and potentially long delay times (24-72 hrs) before detecting acute kidney injury. A kidney function monitor is being developed by MediBeacon, based on transdermally measured fluorescence clearance of the novel fluorescent tracer agent, MB-102. After vascular injection, the agent equilibrates into the extracellular spaces of the body and is cleared exclusively by the kidneys, without being metabolized. Plasma pharmacokinetic (PK) analysis of MB-102 compared to the known GFR agent, iohexol, across subjects with a wide range of chronic kidney disease states, has demonstrated the close equivalence (R^2=0.99) of the GFR derived by the two methods. Transdermal monitoring is accomplished using blue (peak □~450 nm) LED excitation to induce green (peak □~560 nm) fluorescence of MB-102. In a pilot study, the full day fluorescent decay kinetics of MB-102 were shown to be directly related to body-size normalized GFR (tGFR). Achieving accurate GFR assessment from shorter time segments is a primary goal, in order to provide near real-time monitoring of kidney function, for example in hospital intensive care units (ICU). The primary interferents to the tGFR measurement are hemoglobin, melanin, and tissue autofluorescence. The focus of the talk will be on the development of several generations of instruments designed to address these challenges, and their performance during clinical studies to date. Business and regulatory challenges faced along the path toward commercialization of this combination device and agent, will also be briefly described.

Figure 4 – MediBeacon Transdermal GFR Monitor and Agent

COMMERCIALIZATION OF PHOTODISINFECTION TECHNOLOGIES

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BRINGING SFDI TO CLINICAL PRACTICE AT MODULIM

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Key Words: Spatial Frequency Domain Imaging (SFDI), Circulatory compromise, optical imaging

Modulim (previously Modulated Imaging) is dedicated to bringing optical technologies to the clinical for the non-invasive and rapid assessment of tissue health. We have received 510(k) clearance for two medical devices, with an indication to determine oxygenation levels in superficial tissues for patients with potential circulatory compromise. Our devices are currently the only FDA-cleared systems that uses spatial frequency domain imaging (SFDI) as the underlying measurement method. SFDI uses structured illumination to quantify tissue optical properties over large fields of view. SFDI was developed by researchers at the Beckman Laser Institute and Medical Clinic, and a number of labs have continued to publish on the promise of this technology for assessment of tissue health. In this talk, we will share our experiences in translating technology from an academic lab, building the infrastructure to gain regulatory clearance, and the hurdles we face for going to market. Our translation challenges included finding time and money for technology validation, market evaluation, and risk mitigation. Challenges in building infrastructure included implementation of an appropriate quality system, execution of a regulatory strategy, and incorporation of scalable procedures. Of note, our regulatory work included careful choices for component-wise benchtop verification testing along with pre-clinical and clinical validation to establish substantial equivalence of our device to a predicate device. Lastly, we will cover the challenges faced for our go to market device, the Clarifi Imaging System. These challenges include product/workflow fit, reimbursement, and customer support. A common theme that has been true in all of these phases of growth: investment in individuals to enhance and compliment our team strengths.

ADVANCES IN COMPUTATIONAL WIDE FIELD IMAGING

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DESIGN, FABRICATION, AND TEST OF MICRO-OPTICS FOR BIOMEDICAL APPLICATIONS

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SPECTROSCOPIC SINGLE-MOLECULE LOCALIZATION MICROSCOPY (SSMLM)

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Traditional single molecule localization microscopy analyses the spatial distributions of photons emitted by individual molecules to reconstruct super-resolution optical images. To further push the envelope of this imaging technology, we developed spectroscopic single molecule localization microscopy (sSMLM) that is capable of capturing the inherent spectroscopic signatures of photons from individual stochastic radiation events. sSLMLM further improved the spatial resolution of single molecule localization microscopy through spectral discrimination to identify the photons emitted from individual molecules. As a result, we demonstrated a resolution of sub-10 nm without significantly increase the total number of image frames through a novel regression method. Using sSMLM, we demonstrated simultaneous multi-color super-resolution imaging, where the number of fluorescence labels can have largely overlapping emission spectra with only minute differences. In addition, we further investigated intrinsic stochastic fluorescence emission from unstained nucleotides using sSMLM, seeking potential label-free super-resolution imaging

TOO FAR, TOO SMALL, TOO DARK, TOO FOGGY: ON THE USE OF MACHINE LEARNING FOR COMPUTATIONAL IMAGING PROBLEMS

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Key Words: Computational imaging, inverse problems, machine learning.

Computational imaging system design involves the joint optimization of hardware and software to deliver high fidelity images to a human user or artificial intelligence (AI) algorithm. For example, in medical tomography CAT scanners produce non-invasively cross-sectional images of the patient's organs and then medical professionals or, increasingly, automated recognition systems perform diagnosis and decide upon a course of treatment. We refer to this operation of AI as *image interpretation.*

This talk is about a different paradigm where machine learning (ML) is used at the step of image formation itself, *i.e.* for *image reconstruction* rather than interpretation. The ML algorithm, typically implemented as a deep neural network (DNN), is programmed using physically generated or rigorously simulated examples of objects and their associated signals produced on the sensor (or camera.) The training phase consists of adjusting the connection weights of the DNN until it becomes possible, given the sensor signal from a hitherto unseen object, for the DNN to yield an accurate estimate of the object's spatial structure.

The ML approach to solving inverse problems in such fashion has its roots in optimization methods employed long before in computational imaging, compressed sensing and dictionaries in particular. By replacing the proximal gradient step of the optimization with a DNN [K. Gregor & Y. LeCun, ICML 2010], it becomes possible to learn priors other than sparsity, and restrict the object class almost arbitrarily to facilitate the solution of "hard" inverse problems, e.g. highly ill-posed and highly noisy at the same time. Moreover, execution becomes very fast because pre-trained DNNs mostly consist of forward computations which can easily be run at real time, whereas traditional compressed sensing optimization routines are generally iterative. DNN training is time consuming too, but it is only run up front while developing the algorithm; it is not a burden during operation. Unfortunately, however, with the DNN approach some of the nice properties of compressed sensing are lost, most notably convexity.

In this talk we will review these basic developments and then discuss in detail their application to the specific problem of phase retrieval in lensless (free-space propagation) or defocused imaging systems. More specifically, we will investigate the impact of the power spectral density of the training example database on the quality of the reconstructions. We will review a sequence of papers where we first ignored this problem [A. Sinha et al, *Optica* 4:1117, 2017], then improved it in an *ad hoc* way by pre-modulation of the training examples [Li Shuai et al, *Opt. Express* 26:29340, 2018] and finally devised a dual-band approach where the signal is first separated into its low- and high-frequency components, their respective reconstructions are obtained by two DNNs trained separately and then re-composed by a third "synthesizer" DNN [Deng Mo et al, arXiv:1811.07945]. We will explain why each new attempt improves resolution and overall fidelity through progressively more balanced treatment of the spatial frequency spectrum.

We will also discuss implications of this method for phase retrieval under extremely low-photon (too dark) conditions [A. Goy et al, *Phys. Rev. Lett.* 121:243902, 2018] other related inverse problems, e.g. super resolution (too far or too small), and imaging through diffusers (too foggy.)

CAN WE CONTROL HIGHLY COMPLEX AND NONLINEAR OPTICAL SYSTEMS?

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TOWARD A THINKING MICROSCOPE: DEEP LEARNING-ENABLED COMPUTATIONAL MICROSCOPY AND SENSING

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Deep learning is a class of machine learning techniques that uses multi-layered artificial neural networks for automated analysis of signals or data. The name comes from the general structure of deep neural networks, which consist of several layers of artificial neurons, each performing a nonlinear operation, stacked over each other. Beyond its main stream applications such as the recognition and labeling of specific features in images, deep learning holds numerous opportunities for revolutionizing image formation, reconstruction and sensing fields. In fact, deep learning is mysteriously powerful and has been surprising optics researchers in what it can achieve for advancing optical microscopy, and introducing new image reconstruction and transformation methods. From physics-inspired optical designs and devices, we are moving toward data-driven designs that will holistically change both optical hardware and software of next generation microscopy and sensing, blending the two in new ways. Today, we sample an image and then act on it using a computer. Powered by deep learning, next generation optical microscopes and sensors will understand a scene or an object and accordingly decide on how and what to sample based on a given task – this will require a perfect marriage of deep learning with new optical microscopy hardware that is designed based on data. For such a thinking microscope, unsupervised learning would be the key to scale up its impact on various areas of science and engineering, where access to labeled image data might not be immediately available or very costly, difficult to acquire. In this presentation, I will provide an overview of some of our recent work on the use of deep neural networks in advancing computational microscopy and sensing systems, also covering their biomedical applications.

Short Bio

Dr. Ozcan is the Chancellor's Professor at UCLA and an HHMI Professor with the Howard Hughes Medical Institute, leading the Bio- and Nano-Photonics Laboratory at UCLA and is also the Associate Director of the California NanoSystems Institute. Dr. Ozcan is elected Fellow of the National Academy of Inventors (NAI) and holds 40 issued patents and >20 pending patent applications and is also the author of one book and the co-author of >700 peer-reviewed publications in major scientific journals and conferences. Dr. Ozcan is the founder and a member of the Board of Directors of Lucendi Inc. and Holomic/Cellmic LLC, which was named a Technology Pioneer by The World Economic Forum in 2015. Dr. Ozcan is also a Fellow of the American Association for the Advancement of Science (AAAS), the International Photonics Society (SPIE), the Optical Society of America (OSA), the American Institute for Medical and Biological Engineering (AIMBE), the Institute of Electrical and Electronics Engineers (IEEE), the Royal Society of Chemistry (RSC), and the Guggenheim Foundation, and has received major awards including the Presidential Early Career Award for Scientists and Engineers, International Commission for Optics Prize, Biophotonics Technology Innovator Award, Rahmi M. Koc Science Medal, International Photonics Society Early Career Achievement Award, Army Young Investigator Award, NSF CAREER Award, NIH Director's New Innovator Award, Navy Young Investigator Award, IEEE Photonics Society Young Investigator Award and Distinguished Lecturer Award, National Geographic Emerging Explorer Award, National Academy of Engineering The Grainger Foundation Frontiers of Engineering Award and MIT's TR35 Award for his seminal contributions to computational imaging, sensing and diagnostics.