Northeast Symposium on Biomedical Optics 2019

Book of Abstracts

Presentations

Technical Advances in Imaging

All-optical neurophysiology using high-speed wide-area optical sectioning

Vicente Parot (Harvard University)

All-optical stimulation and recording of neural activity could characterize brain function over large areas, but requires compatible optogenetic actuators and reporters, and optical systems for stimulation and optically sectioned imaging in turbid tissue. To stimulate and record activity from thousands of neurons with one photon (1P), we paired a blue shifted channelrhodopsin (eTsChR) with a red-shifted calcium indicator (H2B-jRGECO1a). To image cellular-resolution activity in large areas (4.6 mm FOV) of acute brain slices, we used a digital micromirror device (DMD) to illuminate neighboring sample locations with orthogonal functions of time based on Hadamard codes, and rejected uncorrelated background. To record high-speed neuronal activity (500 Hz), we designed a compressed sensing strategy for Hadamard microscopy, obtaining one optical section every two camera frames. We made functional maps showing that these optogenetic and optical tools provide a powerful capability for wide-area interrogation of neuronal excitability and functional connectivity in acute brain slices.

Optical Coherence Tomography – Frequency Comb Benefits

Norman Lippok (Massachusetts General Hospital and Harvard Medical School)

Optical coherence tomography (OCT) is a powerful three-dimensional, micrometer-scale resolution imaging modality, with impactful clinical applications in ophthalmology and increasing acceptance in cardiology, dermatology and gastroenterology. Unfortunately, similar to other three-dimensional optical imaging methods, conventional OCT excels in controlled environments but is difficult to deploy over large and dynamic fields. This is further hindered by the need for speed yielding signal bandwidths that cannot be detected with electronics. To address these shortcomings, we developed methods to efficiently interrogate sparse scattering fields. Frequency comb sources are used to fold the depth space of interferometric signals thereby superimposing signals from equispaced locations

in a circular manner through optical subsampling. As a result, signal acquisition barriers that have limited speed and field can be avoided. In this talk, I will discuss benefits and challenges of Circular-Ranging OCT and describe new ultrafast frequency comb laser designs that achieve imaging of multi-cm3 fields up to 7.5 volumes per second.

Optics for Global Health

Nucleic acid quantification in Uganda using portable, energy-flexible device

Ryan Snodgrass (Cornell University)

The decentralization of diagnostics in resource limited settings can reduce the time to treatment of many infectious diseases. In Uganda, for example, diagnosis of a common cancer called Kaposi's sarcoma may be unsuccessful because human biopsy samples must travel from rural healthcare clinics to central pathology labs, which compromises the chain of communication with patients. As an alternative to pathology, nucleic-acid-based diagnosis of Kaposi's sarcoma is being pursued. We built a small (1 kg) device that performs nucleic acid amplification using any heat source available, such as sunlight, flame, or electricity – making the device practical to use at both rural clinics and in central laboratories. Fluorescence and turbidity of up to six samples is tracked in real-time to provide quantification of target DNA. Furthermore, the device stores large amounts of heat in a phase change material to maintain proper conditions for the nucleic acid assay even if an energy source is disrupted (e.g. electricity outage, cloud coverage). We deployed many of the devices to Uganda in 2017, where they are still in use today. We discuss how the device works, our field trial to Uganda, and future directions of the project.

Mobile health technologies with applications in medicine

Luis Pacheco (Brigham and Women's Hospital and Harvard Medical School)

The application of mobile devices to healthcare (including smartphones, tablets and wearable devices) is now defined as mobile Health (mHealth). mHealth is increasingly becoming a prominent part of the healthcare system and holds promising for improving patients' access to diagnosis and treatment and for enhancing adherence and self-management of treatment regimens, particularly in resource-limited settings. Smartphone-based biosensing systems perfectly meet the mHealth diagnostics concept, as they can be used in a decentralized manner, have increasing data processing capabilities with low-to-moderate cost, and can be fully integrated with the public-health databases through the internet. At Prof. Hadi Shafiee's Lab (Brigham and Women's

Hospital / Harvard Medical School; https://shafieelab.bwh.harvard.edu/) we have been developing smartphone-coupled mHealth technologies that have successfully been translated to reproductive health applications, including sperm quality analysis and ovulation testing, and also for infectious diseases diagnostics, such as Zika virus detection and HIV viral load quantitation. We employ a highly interdisciplinary approach to develop these technologies, combining microfluidics, nanotechnology, 3D printing, mobile microscopy and artificial intelligence. In this seminar I will present some of these recent technologies that have been developed by our group, with particular focus on the use of mobile phone-based imaging for mHealth diagnostics development.

Wearable and Implantable Optics

Multifunctional polymer-based fibers for the recording and modulation of neural circuits

Marc-Joseph Antonini (Massachusetts Institute of Technology)

Electrical, optical and chemical approaches have been used to manipulate and record neuronal activity in vivo. However, the integration of multiple modalities into a single biocompatible neural probe remains a challenge in the field of neural engineering. Here, we utilize a thermal drawing process to fabricate a multifunctional fiber featuring a microfluidic channel for drug and virus delivery, a tungsten microelectrode for electrophysiological recording and an optical waveguide. By leveraging recent advances in the development of photoswitchable drugs, we can modulate the activity of virally transfected neurons with increased spatial and temporal precision. Combining these technologies into a single device enables one-step chemogenetic experiments in vivo, and simultaneous manipulation and electrophysiological monitoring of the mesolimbic reward system in freely moving mice.

Wearable & Implantable Optical Waveguides

Soroush Shabahang (Massachusetts General Hospital and Harvard Medical School)

The rapid advancements in developing new biomedical devices with optical interfaces necessitates the engineering of new optical waveguides and fiber devices with desired optical, mechanical and biological properties. The future medical applications of optical waveguides include diagnostics, drug delivery, phototherapy, wearable devices and optical sensors. Next generation of medical wearable devices will enable long-term monitoring of patient health, collection of functional data to aid diagnoses, and rapid detection of life-threatening conditions. Implantable optical waveguides with specific

optical, biological and mechanical properties are of great importance in future photomedicine. Optical fiber sensors with different sensing mechanisms can detect small variations in their surroundings. They can potentially cover large areas and provide higher resolution, speed, and sensitivity than their electronic-based counterparts. Multimaterial fibers with temperature and pressure sensors, conductive electrodes and light guiding cores will deliver optical and electrical signals. In this presentation I will review the recent advances in implantable and wearable optical waveguides for biomedical applications and will share some of our recent research in this field.

High optode-density wearable probe for monitoring breast tumor dynamics during neoadjuvant chemotherapy

Sam Spink (Boston University)

We present a high optode-density continuous-wave (CW) wearable diffuse optical device for the investigation of hemodynamic responses of locally advanced breast tumors during neoadjuvant chemotherapy (NAC). The goal of this work is to provide an accessible technology for assessing cancer treatment response in the hospital and home. The device consists of a rigid-flex substrate with 32 LEDs at two wavelengths (750 nm and 850 nm) and 16 detectors. It is highly flexible and can conform to the natural shape of the breast. Measurements on spatially-complex tissue-simulating phantoms were utilized to validate both simpler topographic visualizations and more complex 3-D tomographic reconstructions using the Rytov approximation. The ability to of the probe to capture spatial variation was evaluated by monitoring a channel flow phantom whose nigrosin dye concentration varied within the channel over time. The ability of the probe to capture hemodynamic responses to perturbations in vivo was validated through cuff occlusions and a normal volunteer study employing breath holds. During occlusions (n=2), we identified a mean peak increase in deoxyhemoglobin and decrease in oxyhemoglobin in the forearm of 7.65 ± 1.72 and 4.78 ± 1.22 μM, respectively. From initial volunteer data (n=4 out of 10 planned volunteers), we identified a mean rise in oxyhemoglobin of 1.71 ± 0.89 μM occurring in normal breast tissue following a 30 sec breath hold. A clinical study will commence shortly, and we aim to quantify tumor contrast during breath holds, and assess if treatment response can be determined during NAC.

Posters

1. Digital adaptive optics in optical coherence tomography with phase unstable sources

Sebastián Ruiz-Lopera (Applied Optics Group, Universidad EAFIT, Medellín, Colombia)

We developed a scheme (SHARP) for digital aberration correction in optical coherence tomography (OCT) with phase unstable sources that is based on digital adaptive optics (DAO) and numerical phase-jitter correction, under the notion that local phase stability is sufficient for the deconvolution performed in DAO. SHARP is based on the sample signal itself and does not rely on phase references or custom OCT configurations that acquire phase stable volumes. We demonstrate its applicability in a polygon-laser OCT experiment, achieving successful refocusing at depths up to four times the Rayleigh range. We also present in-vivo skin data where sample motion is addressed and anterior segment data, showing significant enhancement of image quality, particularly when combining SHARP results with state-of-art despeckling technique TNode.

2. A Widefield Mid-Infrared Photothermal Microscope for Chemical Imaging at kHz Frame Rate and Sub-micron Spatial Resolution

Yeran Bai (Boston University)

Mid-infrared photothermal microscopy provides submicrometer spatial resolution with infrared spectroscopic information in a far-field manner. However, its speed is limited by the point scan configuration and tens of seconds was often needed to image a single cell. Here, we push the imaging speed to 1250 frames/s with the presented widefield mid-infrared photothermal microscope. The time-gated virtual lock-in technique was developed to achieve time-resolved imaging with submicrosecond temporal resolution. Living cell chemical imaging was performed at submicrometer spatial resolution. Collectively, the presented widefield mid-infrared photothermal microscope provides a promising way for dynamic sample characterization and high throughput detection.

3. Direct Visualization of Amphotericin B Orientation in Fungal Membrane by Polarization Stimulated Raman Scattering Microscopy

Pu-Ting Dong (Boston University)

For more than 50 years, amphotericin B (amp B) has been the golden standard drug for treating clinical fungal infections. The general understood working mechanism is that amp B has high binding affinity to ergosterol in the cell membrane of fungal cells, causing membrane leakage and cell death. There are two models proposed to illustrate this binding process, one is a barrel-stave model, the other is forming sterol sponge outside the cell membrane. Here, through polarization stimulated Raman scattering microscopy in the fingerprint window, we are able to achieve direct visualization of amp B in a single

fungal cell membrane. And it is found to be orderly distributed in the cell membrane. Later, through comparison of the signal from amp B and CH2 group at different polarization direction, we draw the conclusion that amp B distributes inside the cell membrane parallelly against the phospholipid backbone, supporting the barrel-stave model.

4. Label-free chemical image cytometry reveals metabolic signatures of cancer cells under stress

   Kai-Chih Huang (Boston University)

Cell metabolism is conventionally studied by mass spectrometry or fluorescence microscopy. The former is unable to provide spatial information at the subcellular level, while the latter has low specificity to study metabolic molecules. Additionally, these methods would either destruct the sample or introduce strong perturbations which might alter cell functions. Here, we present multiplex stimulated Raman scattering (SRS) imaging cytometry as a label-free, high-content, high-throughput platform for single-cell analysis. Multiplex SRS imaging allows separation of cellular compartments such as lipid droplets, endoplasmic reticulum, and nuclei from the cytoplasm. Based on these chemical segmentations, over 250 features were generated and analyzed for each cell. Using SRS imaging cytometry, we studied the metabolic responses of human pancreatic cancer cells to starvation and chemotherapy drug treatments. Through statistical analysis of thousands of cells, we unveiled lipid-facilitated protrusion as a metabolic marker for stress-resistant cancer cells. These results highlight our SRS imaging cytometry as a powerful tool for biological discoveries with a high throughput capacity. Our findings also demonstrate the potential of targeting lipid metabolism for selective treatment of starvation-resistant and chemotherapy-resistant cancers.

5. Method of Synthetic Motion for characterizing Single Particle Tracking Microscopes

   Nicholas Vickers (Boston University)

Single particle tracking (SPT) microscopes have enabled many breakthroughs in biology including understanding both molecular motor function and pathways of viral infection. A major challenge to further developing SPT microscopes is that no existing testing protocol provides a known ground truth. Simulated data have been used exhaustively in the analysis of SPT data analysis algorithms and software, however, it remains to compare different SPT microscope hardware experimentally. One method is synthetic motion, where a fluorophore fixed to a slide is moved along a realization of a stochastic motion model, such as Brownian motion, using a piezo actuated microscope stage. This motion is repeatable and known, allowing direct comparisons, and characterization of microscopes. The ability of the stage to follow the molecular motion is limited by limitations of actuator bandwidth, slew rate, and quantization. Fortunately, a feedforward-feedback control system can mitigate these allowing for a powerful tool in SPT microscope development.

6. Remote-focusing volumetric chemical microscopy for in-vitro and in-vivo label-free imaging

   Peng Lin (Boston University)

Volumetric chemical imaging enables a three-dimensional (3D) measurement of the molecular distribution and concentration in a complex biological system. Stimulated Raman scattering (SRS), as a specific chemical imaging technique, can quantify a brand range of molecules at high-speed in a label-free manner. To realize 3D-SRS imaging, a typical method of axially scanning the focus by moving the objective has limited speed and can introduce optical aberrations and sample agitation. Here, we propose a remote-focusing volumetric SRS microscopy with a MEMS deformable mirror. The new method obtains 3D imaging without mechanically moving of the objective and can correct the system aberrations. We demonstrated the microscopy in in-vivo and in-vitro biological samples.

7. Shortwave Infrared Spatial Frequency Domain Imaging for extracting water and lipid concentrations from biological samples

   Anahita Pilvar (Boston University)

Spatial Frequency Domain Imaging (SFDI) is a non-contact and label-free diffuse optical imaging tool that is used to quantify optical properties of biological samples. Measured absorption values can then be used to extract molar concentrations of light-absorbing chromophores in tissue. Most current SFDI systems utilize visible and near-infrared wavelength bands, which are ideal for quantifying oxy- and deoxyhemoglobin concentrations. We have recently extended SFDI to the Shortwave Infrared (SWIR) where water and lipid have distinct absorption features. We will present the performance results of our SWIR-SFDI system as well as results from several potential new application areas enabled by the unique features of our system. The extraction accuracy and precision for water and lipid extractions will be presented. We will present in vivo results comparing SWIR-SFDI to frequency-domain Diffuse Optical Spectroscopy. We will also present a two-layer look-up-table inverse model that accounts for the effect of skin during clinical imaging.

8. Staphyloxanthin-Targeting Phototherapy Platform for Methicillin-Resistant Staphylococcus aureus Infections

   Jie Hui (Boston University)

Confronted with the rapid evolution and dissemination of antibiotic resistance, there is an urgent need to develop alternative treatment strategies for drug-resistant pathogens. Here, triggered by an accidental discovery, we present an unconventional treatment platform for S. aureus infections via photo-disassembly of its functional membrane microdomains. The photo-disassembly of microdomains is based on effective photolysis of staphyloxanthin, the golden carotenoid pigment that gives its name and an antioxidant

that shields S. aureus from reactive oxygen species (ROS) attack. The nonlinear staphyloxanthin photolysis kinetics suggest a short-pulsed laser for dramatically improved photolysis speed and efficiency. Upon pulsed laser treatment, cell membranes are found severely disorganized and malfunctioned to defense antibiotics and ROS, as unveiled by membrane permeabilization, membrane fluidification, and detachment of membrane proteins. Consequently, our photolysis approach increases susceptibility and inhibits development of resistance to a broad spectrum of antibiotics including penicillins, quinolones, tetracyclines, aminoglycosides, lipopeptides, oxazolidinones, and ROS.

9. Ultrasound induced single cell modulation enabled by a fiber-based optoacoustic emitter

   Linli Shi (Boston University)

Focused ultrasound has attracted great attention in minimally invasive therapy, drug delivery, gene transfer, and brain stimulation. Nevertheless, the poor spatial resolution due to the acoustic diffraction limit (~mm) and the large device size (~cm) severely compromised the capability of pinpointing a specific region. To break the diffraction limit, enabling broad and new biomedical studies, we developed a fiber-based optoacoustic emitter (FOE) based on nanoparticle-polymer matrix as a point ultrasound source (20 micron Dia.). We further demonstrate its application in single cell delivery of small molecules, single neuron stimulation as well as direct axon stimulation, which is not possible for any existing ultrasound techniques. It holds promise for revealing the controversial mechanism of ultrasound induced neuron stimulation, as well as providing a non-contacting approach for broad and new biomedical studies including drug delivery, gene transfection, etc.

10. Deep learning for OCT angiogram vectorization

    Sabina Stefan (Boston University)

Optical coherence tomography (OCT) is a rapid, label-free, high resolution imaging tool that is becoming increasingly popular and useful for neuroscientific study. OCT may be particularly useful for the analysis of vascular networks, which are visualized based on dynamic scattering in vessels due to moving blood cells. One promising application of OCT is in the lifespan tracking of microvascular alterations in neurological diseases such as Alzheimer's Disease, without the need for contrast agents, additionally allowing measurements of blood flow in penetrating arterioles and venules using Doppler OCT as well as blood flow velocity in capillaries. However it is not realistic to semi-manually segment and vectorize a large number of longitudinal OCT datasets using existing methods. Additionally, many methods applied to the segmentation of the vasculature using other imaging modalities (for example two-photon microscopy) are not as successful on OCT angiograms due to the effects of multiple-scattering which results in projection artifacts or “tails”. To combat this issue, we have trained a deep convolutional neural network to automatically segment OCT angiograms, using labeled data consisting of angiograms that are fully segmented and manually corrected. Application of our

network to unseen test data of standard angiograms yields promising results, performing significantly better than standard methods of vessel segmentation without the need for empirical parameter optimization. Segmented data can now be vectorized more easily and accurately, making it possible to quantitatively investigate questions concerned with angioarchitecture, blood flow and neurovascular coupling.

11. Rapid beam scanning restores redox contrast in high repetition rate transient absorption of cytochrome c

    Erkang Wang (Colorado State University)

We are developing a label-free imaging technique with the ability to distinguish redox states of cytochrome c based on pump-probe microscopy. In the proposed technique, a pair of the ultrafast pump and probe pulses, with a center wavelength of 530 and 490 nm respectively have been used to observe excited electron dynamics with sub-picosecond temporal resolution. To lower the heat accumulation induced by the laser focal point, a scanner with a fast line scanning rate of 3.5 kHz has been utilized. Thus the fast detection rate of lock-in amplifier (LIA) is required for high-resolution imaging. With a software-based adaptive filter, the detection rate is increased by lowering the noise floor of the conventional LIA. The transient absorption responses from cytochrome c solution and mouse muscle, as well as the related electronic dynamics, will be presented. Moreover, the enhanced SNR benefited from the adaptive filter will be presented.

12. Multi-parameter polarization-sensitive optical coherence tomography for improved burn depth determination

    Taylor Cannon (Harvard-MIT Health Sciences and Technology)

There is a need for better assessment of injury depth to improve treatment guidance for burn patients. Polarization-sensitive optical coherence tomography (PS-OCT) leverages the polarization properties of skin and has been proposed as a potential solution. While the network of collagen in healthy skin is birefringent and causes depolarization, burned skin lacks these properties and provides PS-OCT an opportunity to evaluate burn depth. We augment the birefringence signal by measuring depolarization, a function of collagen organization, and intensity attenuation, a function of tissue microstructure. We hope that this work will be useful for accurate burn depth determination in clinical settings.

13. Preferential Uptake Dynamics of Single-Walled Carbon Nanotubes in Live Macrophages

    Maha Yaqoob (Laser Biomedical Research Center, MIT)

There has been significant interest in single-walled carbon nanotubes (SWCNTs) for targeted drug delivery and optical as well as electronic sensors. In the past, we studied cellular uptake of different-species SWCNTs, wrapped by an identical DNA sequence, in live macrophages. We found no evidence of preferential uptake of SWCNTs. Recently,

we have conducted a similar study with identical SWCNTs, wrapped by two distinctive DNA sequences (GT6 and GT30). We found that SWCNTs wrapped in GT30 DNA sequence had higher uptake rate in live macrophages. In this poster, we will present our latest results on SWCNT uptake dynamics as well as their distribution maps inside the macrophages at different time points for the two DNA sequences. For label-free measurement of SWCNT uptake, we have employed a custom-built high-speed confocal Raman microscope to acquire movies of both nanotube-based as well as cell-intrinsic Raman signals.

14. Effect of anesthetics (isoflurane vs. α-chloralose) on spontaneous neural activity in the rat neocortex

Kwangyeol Baek (Martinos Center for Biomedical Imaging, MGH)

Spontaneous brain activity has been widely investigated in functional connectivity studies using anesthetized animals. Here, we explored the resting state rat neocortex using bihemispheric laminar electrophysiological recordings of the local field potential (LFP) to investigate how varying types and doses of anesthetics influence the LFP activity and functional connectivity. In both awake and light sedation states, continuous LFP oscillations in gamma and delta/theta bands were observed. Delta/theta bands elevated as the isoflurane dose increased while the burst suppression pattern of activity emerged only when the isoflurane dose was 1.5% or higher. On the other hand, the spontaneous LFP activity under anesthesia with α-chloralose was characterized by continuous large, slow oscillations. Sensory-evoked response intensified with α-chloralose, resulting in a more visible response in the ipsilateral somatosensory cortex than it did in the awake state. Strikingly, α-chloralose increased the interhemispheric correlation in a layer-specific manner, whereas isoflurane induced nonspecific global correlation.

15. *In Vivo* Optical Monitoring of Intraocular Pressure in Boston Keratoprosthesis

Pui-Chuen "Wallace" Hui (Massachusetts Eye and Ear and Harvard Medical School)

We demonstrate direct integration of a miniaturized fiber-optic Fabry-Perot pressure sensor (PS) into an artificial cornea, named Boston Keratoprosthesis (B-KPro), to facilitate quantitative intraocular pressure (IOP) monitoring and improve glaucoma management. The sensor-integrated B-KPros are illuminated and probed with an external multimode fiber and an optical correlator module for white-light interferometry. To achieve efficient optical coupling robust against saccades and tremor, we implemented a novel fiber-optic self-alignment scheme by fitting NdFeB micro-magnets over the probing fibers and the sensors. The device was evaluated in vitro to characterize the long-term stability and in vivo by longitudinal IOP measurements in rabbit eyes. PS-endowed B-KPros were implanted in 6 New Zealand White rabbits, which were followed for 6 weeks with weekly IOP measurements validated against intracameral manometry (IM). Our preliminary demonstration shows promises in improving glaucoma management in KPro patients via IOP-sensing KPro.

16. Neural Networks for In Situ Detection of Glioma Infiltration Using Optical Coherence Tomography

Ronald Miguel Juarez Chambi (Texas A&M University)

In brain cancer surgery, it is critical to achieve complete cancer resection without compromising non-cancerous tissue. Various technologies, such as MRI and CT, have made major contributions; however, they do not provide quantitative, real-time and three-dimensional (3D) continuous guidance. Optical Coherence Tomography (OCT) is a non-invasive, label-free, real-time, high-resolution imaging modality that has been explored for glioma infiltration detection. We have developed an automated, real time, in situ, detection method of glioma infiltration using neural networks in high-resolution volumetric OCT brain images. This computer-aided detection (CAD) system overcomes major challenges faced by previous methods that rely on estimating tissue optical properties (e.g. attenuation coefficient) from the OCT signal. An independent blind validation of the CAD system showed auspicious results (sensitivity, specificity: ~90 %) for detecting glioma-infiltrated tissue with high spatial resolution (~16 μm laterally); thus, this method is well-suited to be implemented in an OCT-based CAD system for real-time applications.

17. Development and applications of the dual-slope technique in near-infrared spectroscopy

Giles Blaney (Tufts University)

We present a method that combines two slope measurements of either intensity or phase as a function of source detector distance. In the simplest case this can be done with two light sources and two detectors arranged symmetrically to achieve two measurements of either intensity or phase slope. The combination of slopes results in what we term the dual-slope measurement. This dual-slope has the advantage of reduced sensitivity to the surface and preferential sensitivity to deeper layers with a semi-infinite diffuse medium. We show results applying dual-slope to measurements of hemodynamics in human brain and skeletal muscle. With the aim of extracting blood flow measurements representative of deeper hemodynamics.

18. Differentially Polarized Laparoscopy (DPL) Imaging for Improving Visibility of Peritoneal Metastases

Ahmed Gado (Tufts University)

In 2019, 1.7M new cases of cancer are projected to occur. Determining whether or not cancer lesions occur in the peritoneum is an important clinical marker for cancer metastasis. For that purpose, clinicians use laparoscopy to screen the peritoneal cavity for cancer lesions. However, the sensitivity and specificity obtained with conventional laparoscopy needs improvement. We propose differentially polarized laparoscopy (DPL) as a modality that can meet that need. In this abstract, we report on the performance of DPL on artificial tissue phantom samples. We demonstrate the potential advantage of

DPL compared to conventional imaging in its ability to derive contrast from scattering with little dependence on the absorbance or thickness of the sample. This optical behavior is potentially helpful for some clinical applications.

19. *In Vivo*, Label-Free Optical Detection of Circulating Tumor Cell Clusters Using Back Scatter Flow Cytometry

Nilay Vora (Tufts University)

The majority of cancer related deaths can be attributed to tumors metastasizing to distal organs such as the brain, lungs, and bone. As tumors metastasize, circulating tumor cells (CTCs) and rare circulating tumor cell clusters (CTCCs), which can range in size from 2-20 cells, are introduced to the bloodstream. CTCCs, in particular, have shown an increased ability to survive in the bloodstream and are more likely to lead to a metastasis than individual CTCs. Due to CTCCs increased metastatic potential, there is a growing need to detect these rare events in the bloodstream for improved diagnosis, stratification, and treatment of cancer patients. This study utilizes a configured confocal back scatter and fluorescence flow cytometer (FIVFC) to optically detect CTCCs in vivo. Using NOD/SCID mice injected with MD-MBA 231 CTCCs, this study establishes the sensitivity and specificity of the system and evaluates the potential of the custom FIVFC for CTCC detection.

20. Label-free, optical, morpho-functional cancer biomarkers

Christopher Polleys (Tufts University)

Half a million new cases of cervical cancer are diagnosed each year. The annual cost of screening and treating cervical pre-cancerous lesions is $6 billion in the US alone. Current methods of colposcopic assessment yield <50% specificity and 95.6% sensitivity of detection for high-grade squamous intraepithelial lesions after the acquisition of three biopsies. Optical methods provide a non-invasive diagnostic procedure capable of sampling multiple colposcopically abnormal regions of the cervix in real-time. Two-photon excited fluorescence (TPEF) offers intrinsic depth sectioning and high detection efficiency necessary for yielding high-resolution optical sections of 3D epithelial tissues. TPEF imaging of NADH and FAD provides diagnostically useful information regarding changes in morphological, biochemical, and mitochondrial dynamics. In this study, we demonstrate the translational impact of label-free TPEF for the automated classification of healthy, low-grade, and high-grade squamous intraepithelial lesions using the combined morpho-functional information of 25 freshly excised human cervical tissues.

21. Photosensitizer loaded photoacoustic nanodroplets for oxygen enhanced photodynamic therapy

Marvin Xavierselvan (Tufts University)

Photodynamic therapy (PDT) utilizes light and photosensitizer to generate cytotoxic species such as singlet oxygen. The effectiveness of PDT, especially the type II pathways, depends on the availability of oxygen in the vicinity. Many tumors lack oxygen, making them resistant to PDT and other therapies. Hence there is need to enhance the oxygen content of tumors while delivering sufficient photosensitizer dose. Building on our previous work using perfluorocarbon nanodroplets to deliver dyes, here we employed them as a carrier for oxygen along with photosensitizer Benzoporphyrin derivative. We continued to use the photoacoustic imaging contrast agent Indocyanine Green in the construct to enhance imaging signal. Photoacoustic imaging was chosen as it can provide information about the blood oxygen saturation and drug concentration simultaneously at ultrasonic resolution in tissue penetration depths superior to optical imaging alone. Through in vitro experiments, we confirmed that the nanodroplets could deliver oxygen and generate singlet oxygen in hypoxic conditions. Next, we tested the delivery of oxygen, drug, and dyes within nanodroplets in a murine model bearing subcutaneous FaDu tumors using multi-wavelength photoacoustic imaging. We validated our imaging results with an oxygen sensing probe where a 9.1 (+/- 1.8 SEM) fold increase in oxygen content inside the tumor was observed immediately after systemic injection of the nanodroplets. Finally, we performed in vivo studies on subcutaneous FaDu tumors treated with varying types of nanodroplets and drug light intervals for PDT. In summary, this new nanodroplet platform has the capability to significantly enhance PDT while providing patient-specific treatment options.

22. Non-contact detection of hemodynamic and perfusion properties of skin with multi-layer, GPU-accelerated Monte Carlo modeling

Wyatt Austin (University of Maine)

Here we combine Spatial Frequency Domain Imaging (SFDI) and Laser Speckle Contrast Imaging (LSCI) to explore both hemodynamic and perfusion states of skin. The goal of this work is to detect physiological changes associated with the progression of diseases such as diabetic polyneuropathy and peripheral arterial disease in hopes of developing a non-contact method for early detection. SFDI is a widefield diffuse optical technique sensitive to oxy- and deoxyhemoglobin composition of tissue, while LSCI has the advantage of quantifying tissue perfusion with random interference patterns detected from doppler-shifted photons. In this work, we have developed a GPU-accelerated, multi-layer Monte-Carlo-based model to address both SFD and LSC inverse problems. With this nimble, tissue-specific lookup table generation method, optical properties from the SFD are used to recover hemodynamic properties of skin while also informing an LSC model to estimate layer-specific flow dynamics.

23. Imaging Zebrafish with Duchenne Muscular Dystrophy using Second-Harmonic Generation to Evaluate Myosin Structure

Jordan Miner (University of Maine, Orono)

Duchenne muscular dystrophy (DMD), an incurable disease that causes weakness and loss of muscle mass, alters the sarcomere structure. Using second harmonic generation (SHG) imaging, the sarcomere length of a DMD zebrafish model can be evaluated to determine the impact of four different exercise regiments: endurance, hypertrophy, strength and power. Preliminary results demonstrate a reduced sarcomere length in DMD zebrafish when compared to wild-type zebrafish. Additionally, DMD zebrafish exercised with neuromuscular electrical stimulation (NMES) to emulate endurance strength training show an increase in sarcomere length when compared to non-stimulated DMD zebrafish. Overall, this study will effectively combine SHG imaging with the use of zebrafish to optimize muscular myosin evaluation, furthering our knowledge of DMD.

24. Ultrasound elastography of arterial wall: an acoustoelasticity based approach

Guoyang Li (Wellman Center for Photomedicine (MGH) and Harvard Medical School)

Characterizing arterial stiffness is of clinical importance and has attracted considerable attentions. In this study, acoustic radiation force and ultrafast ultrasound imaging are utilized to induce and track the guided axial waves (GAWs) in common carotid arteries. An acoustoelastic model is built to address the effect of blood pressure on dispersion relations of GAWs.

25. Development and evaluation of a trans-cutaneous tissue oxygenation wearable device

Juan Pedro Cascales (Wellman Center for Photomedicine (MGH) and Harvard Medical School)

The direct measurement of tissue oxygen concentration (pO2), in particular transcutaneous oxygen monitoring (TCOM), is not only immensely useful in trauma and surgical settings to record the oxygen concentration but could also be applied to determine real-time muscle oxygenation and hence lactate threshold, critical in building and maintaining performance. Currently available devices usually monitor proxies of pO2 such as the oxygen saturation of hemoglobin in underlying tissue (SpO2 or StO2 respectively) and blood flow. However, current TCOM devices require time-consuming and frequent bedside calibration, precise placement, and well-trained operators. We have developed a wireless (Bluetooth or Wi-Fi), light and ergonomic optical TCOM wearable which requires no calibration, minimal user training, no application of heat to the measurement site, no exogenous agents, etc. Our device is based on an oxygen-sensing polymer film, embedded with metalloporphyrin-based molecules which display intense, red phosphorescence emission inversely proportional to the oxygen partial pressure.

26. Forward Stimulated Brillouin Scattering Optomechanical Fiber Sensor

    Desmond Chow (Wellman Center for Photomedicine (MGH) and Harvard Medical School)

Forward stimulated Brillouin scattering (FSBS) is an opto-acoustic effect involving interaction of light with transverse acoustic eigenmodes in an optical waveguide, which can be stimulated by electrostriction. The FSBS response can be observed by measuring the refractive index modulation caused by transverse acoustic waves. For sensing, liquid acoustic impedance measurement using FSBS has been demonstrated with an uncoated single mode fiber (SMF). Transverse acoustic waves in the fiber cladding interact directly with the fiber surroundings through boundary reflection and the acoustic decay rate is retrieved to calculate the acoustic impedance of the surrounding liquid. Here, a frequency sweeping method is used, owing to the contrast between the long inertial response of FSBS and the instantaneous nature of Kerr effect, a flexible and undistorted FSBS spectral linewidth measurement is demonstrated.

27. Highly Sensitive and Reliable Plasmonic Nanoparticle-based Digital Cytometry for Quantification of MUC16 Binding on the Surface of Leukocytes

    Sinyoung Jeong (Wellman Center for Photomedicine (MGH) and Harvard Medical School)

We recently developed a digital cytometric assay using plasmonic nanoparticles to quantify the bound ovarian cancer marker (CA125) on single cells. We demonstrate the quantification capability of our novel digital cytometry method by discriminating the different MUC16 binding levels on the PBMCs between healthy donors and EOC patients and tracking MUC16 on the EOC patient's PBMCs over 17 months.

28. Polarization sensitive μOCT tethered capsule endomicroscopy for investigation of eosinophilic esophagitis

    Andreas Wartakn (Wellman Center for Photomedicine (MGH) and Harvard Medical School)

The high cost and inconvenience of endoscopic biopsy fundamentally limits the diagnosis of eosinophilic esophagitis (EoE), motivating the need for a well-tolerated and inexpensive tool assessing esophageal eosinophil count and sub-epithelial remodeling. Here, we introduce an advancement of our laboratory's optical coherence tomography (OCT) tethered capsule endomicroscopy (TCE) technology that incorporates micro-OCT (μOCT) at ~1 μm axial and ~2 μm lateral resolution, and polarization sensitive (PS) image contrast. We present first ex vivo bench-top imaging results, in addition to the design of our PS-μOCT TCE instrument and imaging probe.

29. Real-time oxygenation and force sensing toolkit for optimal tourniquet application: Development of a “smart” emergency tourniquet

John Nguyen (Wellman Center for Photomedicine (MGH) and Harvard Medical School)

Emergency tourniquets are designed to apply circumferential pressure around traumatic limb injuries and to stop severe arterial bleeding prior to definitive care. Its use by properly trained and equipped military personnel has helped to dramatically reduce battlefield deaths, and it has recently gained popularity in the civilian sector with just-in-time point-of-injury prehospital use increasing by a tenfold over the past decade. However, even trained individuals can find it difficult to assess proper tourniquet application. Here we present a real-time tissue oxygenation and capacitive force sensing toolkit that can be combined into a single device capable of integrating with preexisting standard-of-care military tourniquets. The easy-to-use device allows for the real time monitoring of tissue oxygen and applied force in order to assess adequate tourniquet application and track limb viability for triage even if patients are noncompliant or nonresponsive.