Illuminating Biology from Benchtop to Bedside: Biophotonics across Energy, Space, and Time

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Abstract

Biophotonics refers to the use of light to image, probe, manipulate or modify biological systems. Over the past decade, the use of biophotonics methods has become increasingly pervasive throughout the biomedical sciences. We present the current research activity from our IGERT trainees that highlights the utility of biophotonics methods across energy, space, and time to address a diversity of biomedical issues. In one project we are developing a non-linear imaging system capable of generating sub-micron resolution, 3D images of biological structures in under a minute. This device will be used to study collagen fiber nanostructure. A second project is investigating the use of highly-focused laser pulses to generate microscale cavitation bubbles to study cellular mechanotransduction with potential applications to drug screening. In a third research project we are developing novel bioluminescent probes using *Gaussia* luciferase that will be capable of providing dynamic information regarding cell-cell interactions. This technology will be used to generate images of tumor cell interaction on the micrometer scale on the order of a minute.

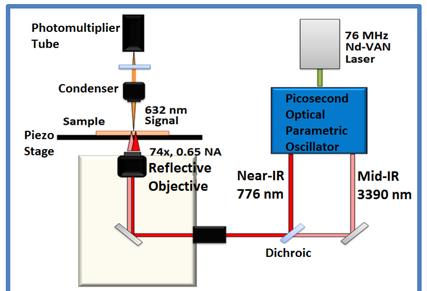
In addition to basic science investigations, our IGERT group is also developing novel biophotonics technologies for clinical translation. We are developing a long-range optical coherence tomography system capable of generating 3D images at 50 frames per second with a resolution of 10 microns, which will be used in the clinic to map the anatomy and structure of the human airway to evaluate sleep apnea. A second clinical translation project focuses on the development of spatial frequency domain imaging (SFDI) that provides wide-field functional images of biochemical tissue compositions on the order of a minute with millimeter resolution. SFDI will be used in the clinic to monitor kidney health during partial nephrectomy procedures.

time

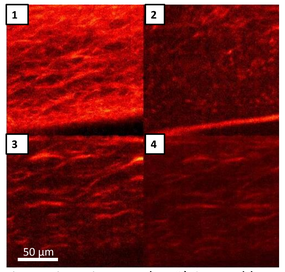
Sum frequency generation microscopy imaging of corneal collagen (J.C. Hsu)

The cornea of the eye is responsible for two-thirds of its refractive power and is composed primarily of type I collagen. Arrangement of these collagen fibers is closely related to corneal function, as differences have been observed in healthy and dystrophic cornea. However, the relationship between minor changes in the corneal lamella and corneal function is unclear. We are developing a sum frequency generation microscopic imaging method to provide high resolution, specificity, and contrast to examine the structure-function relationship of corneal morphology.

Vibrationally-resonant sum frequency generation(VR-SFG) is a second-order nonlinear optical effect described by $\omega_1 + \omega_2 = \omega_3$, where ω_1 is in resonance with the vibrational mode of the molecule. Its sensitivity to noncentrosymmetric biomolecules, including collagen, makes it ideal for this study. We use a VR-SFG to image the structure of a 100 µm thick hawk cornea slice. The nonlinear technique provides imaging within a minute and 3D focusing with submicron resolution. Additionally, the vibrational resonance in the mid-infrared region specifically enhances the signal from the molecule of interest, allowing us to obtain label-free imaging.



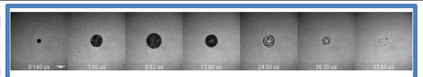
VR-SFG Instrument. Setup utilizes picosecond pulses and collinear excitation that increase signal and reduce sample damage. Excitation beams are focused by a reflective objective, and the signal is collected by a photomultiplier tube.



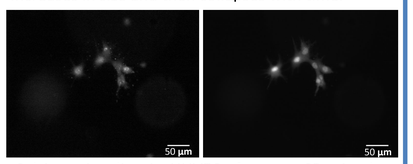
Preliminary imaging results. 1) Second harmonic generation image. 2, 3, and 4) SFG with varying beam polarizations. The high contrast in the polarization study confirms that SFG is an ideal tool for further corneal collagen study.

Mechanotransduction in HUVECs using Laser-Induced Optical Breakdown (J.C. Luo)

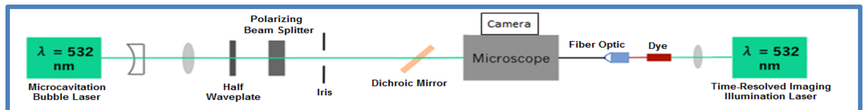
Cellular function is known to be sensitive to mechanical forces via mechanotransduction. Such forces arise from the extracellular matrix environment as well as from other cells via cell-cell interactions. Mechanical forces may affect cellular processes by activating signaling pathways, altering homeostasis, changing function, and influencing fate. We are developing a novel means to initiate and study mechanotransduction of cell signaling pathways using laser-generated microcavitation bubbles (µCB). µCBs are created by the phenomena of laser-induced optical breakdown and involves the formation of plasma in an aqueous medium. Plasma vaporization and subsequent expansion results in a µCB. In this study, Human Umbilical Vein Endothelial Cells cultured in 3D fibrin gels can be perturbed by the formation of a µCB. The µCB induces matrix deformation in the fibrin gel and produces considerable strain on the cells allowing activation of mechanotransduction pathways.



Time series Montage of µCB formation. The µCB is formed after initial laser fire where a shockwave can be observed within nanoseconds. Bubble increases in size and then collapses with time.



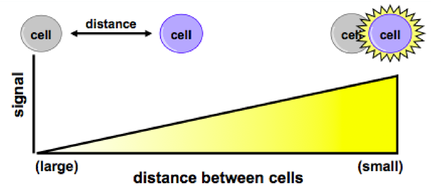
Calcium signaling in HUVECs from a µCB stimulus. HUVECs are observed to release Ca^{2+} from the endoplasmic reticulum following µCB generation. Images on the left and right corresponds to HUVECs before and after being perturbed by a µCB.



µCBs are created by a 532 nm pulsed laser directed to the microscope and focused in the sample. Observation of the bubble is achieved by the technique of time-resolved imaging. Illumination laser pumps a fluorescent dye and the resulting light is directed to the microscope via a fiber optic. Images were acquired using an iCCD camera.

Expanding the bioluminescent tookit for *in vivo* imaging (J.R. Laird)

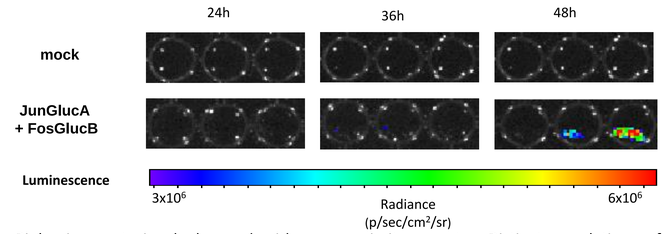
Target recognition and cell-cell contacts are crucial to immune function, yet there are no practical methods to assay such interactions in whole organisms. We aim to develop a general method for studying cellular interactions *in vivo* that blends the sensitivity of bioluminescence imaging with the spatial resolution of histology.



Visualizing cellular proximity with optical reporters. As the distance between two cells decreases, the signal output increases.

In the proposed method, one half of *Gaussia* luciferase is secreted from one population of cells, while the other half is secreted from a second population of cells. A luminescent signal is produced when the two halves bind to form a functional luciferase. To "trap" the interaction between the luciferase fragments, we are using the tight-binding leucine zipper proteins Fos and Jun. Preliminary data suggest that the luciferase halves can be secreted and combined in the extracellular space. In the near future, we plan to test the distance dependence of the optical signal.

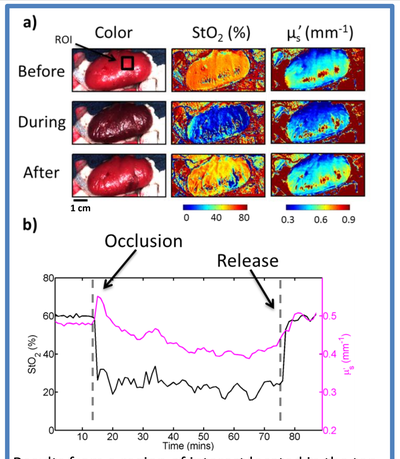
This type of cellular proximity reporter system will accelerate the development of improved cellular therapies, and facilitate studies of cell-cell interactions involved in stem cell differentiation, T cell activation, and other physiological processes.



Bioluminescent signal observed with our proximity reporter. Distinct populations of HEK293 cells expressing two different halves of Gaussia luciferases produce light when mixed. (With Krysten Jones)

Quantifying kidney health during partial nephrectomy (K.P. Nadeau)

Accurate determination of the severity and extent of renal injury during renal artery clamping is one of the major challenges all urologists face during a partial nephrectomy. Currently there are no surgical guidance tools available to monitor kidney tissue health during ischemia. Moreover, there is no means to evaluate the exact time point at which ischemic reperfusion injury (IRI) becomes irreversible. This has resulted in a critical need to understand the pathophysiology of IRI, that would allow the surgeon to make intra-operative adjustments to preserve maximal function of the kidney and minimize renal IRI. We present data obtained during arterial occlusion of a porcine model using spatial frequency domain imaging (SFDI) and demonstrate its ability to provide quantitative, absolute values of oxygen saturation (StO_2) and the reduced scattering coefficient (μ_s') of the kidney during renal arterial occlusion.



Results from a region of interest located in the top-center portion of the kidney (a), corresponding to a field of view of roughly 1x1 cm. Results for tissue oxygen saturation (StO_2) and the reduced scattering coefficient (μ_s') are shown

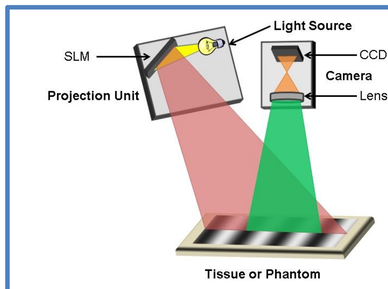


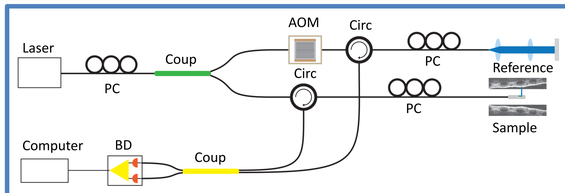
Illustration of SFDI *instrument*. *Patterned* light is impinged on a sample using a light source coupled with a spatial light modulator (SLM) inside a projection unit. The diffusely reflected light is then coupled to a lens and detected by a charge-coupled device (CCD) inside a camera.

StO_2 is used as a metric for tissue metabolism. Upon arterial occlusion, the StO_2 drops rapidly. Upon reperfusion, we see a rapid increase in StO_2, recovering to the baseline level of 60% within 10 minutes.

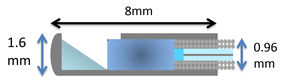
μ_s' could potentially be used to determine tissue health. Throughout the arterial occlusion period, μ_s' decreases in a linear fashion. After an hour, μ_s' is approximately 0.4 mm^{-1}, which corresponds to a 20% decrease during occlusion. This decrease in μ_s' may be linked to cellular inflammation and breakdown due to ischemia.

Mapping the upper airway with long range optical coherence tomography (J.C. Jing)

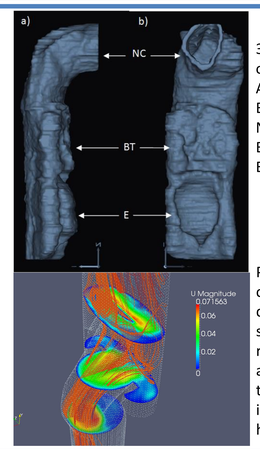
The human airway provides critical daily functions in breathing, eating and phonation. Airway obstruction can lead to disorders such as sleep apnea or airway stenosis. Presently there are limited means to provide structural and anatomical information on the upper airway without the risks of ionizing radiation or sedation. Endoscopic long range optical coherence tomography (OCT) enables ionizing free high resolution cross-sectional optical imaging of biological tissue and can potentially address these needs. We developed a high speed long range endoscopic Fourier domain OCT (FDOCT) system capable of non-invasive real time imaging providing information such as size and shape of the human airway within minutes.



In our OCT system, light from the sample arm images the airway. A 150 MHz AOM is placed in line with the reference arm and the causes a carrier frequency of the resulting interference fringes. This allows for alias free imaging over a longer range. The signal is sampled by a 12 bit ADC at 1 GHz and shifted down to baseband for OCT processing.



A grin lens focuses the exiting light which is directed into the airway via a prism. The entire probe is rotated proximally and translated to achieve a 3D scan of the airway.



3D reconstruction of human Airway.
A) Profile View.
B) Front on View.
NC) Nasal Cavity.
BT) Base of Tongue.
E) Epiglottis

Preliminary computational fluid dynamics simulations. Red regions indicate areas of highly turbulent flow indicating regions of high obstruction

energy

space