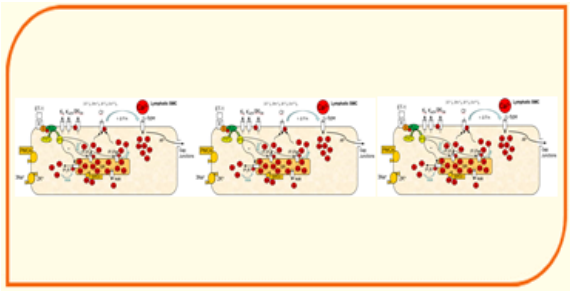
Table of Contents

1

Multiscale modeling of lymphatic vessels: From ion channel activity to lymph transport

A.Moshkforoush, A. Kapela, N. Tsoukias*

Immune function, homeostasis of different fluids in the body, production of antibodies and lymphocytes, and filtering out toxins and macro-organisms are but a handful of important roles carried out by the highly complicated lymphatic system. The failure of this system to fulfill its roles will result in irreparable damage to the regular function of the body. Lymphedema for instance, which has been the focus of extensive research during the past few decades, is one the most well-known debilitating diseases associated with the malfunction of the lymphatic transport. This proposed research outlines a multiscale computational model of collecting lymphatic vessels in which the transport function of lymphatic system, in particular collecting lymphatics, at macroscale level can be analyzed by integrating underlying mechanisms at cellular and molecular levels. To the best of our knowledge, this proposed model is the very first attempt that synergistically takes into account the biomechanics of the lymphatic vessel wall, fluid dynamics, and the electrophysiology of different ionic species in the cells constituting the lymphatic vessels.

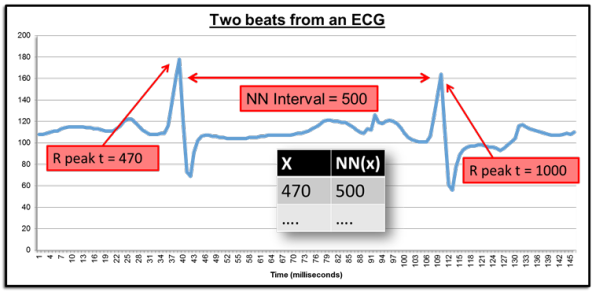


2

Multimodal Analysis of Endotracheal Suctioning in the Pediatric Intensive Care Unit

Teshaun Francis1; Wei-Chiang Lin12*, PhD; Balagangadhar Totapally2, MD Florida International University1 and Nicklaus Children's Hospital2

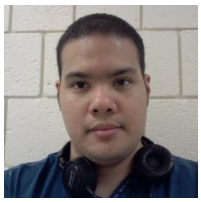
While anaesthetized in the Intensive Care Unit, a patient cannot breathe normally without assistance from a mechanical ventilator. Pulmonary secretions drain to the alveoli and must be excavated through a method called endotracheal suctioning. Without the removal of these fluids the patient is at risk of developing pneumonia, pulmonary edema, or other respiratory diseases which will undoubtedly impede his or her recovery. The impact of the suction technique on the patient's body, however, cannot be fully understood without continuous multimodal monitoring of various physiological signals and the subsequent analysis of these signals. The goal of this research is to obtain an understanding of the physiological changes a patient undergoes when prescribed endotracheal suction.



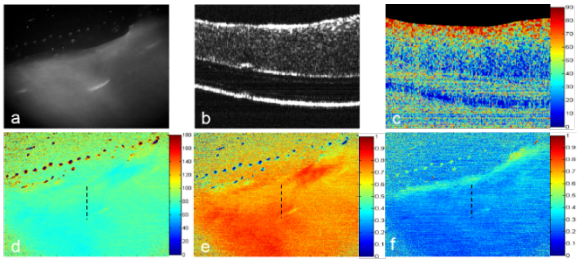
3

Imaging birefringent tissues using polarization-sensitive optical coherence tomography and Mueller matrix imaging.

Joseph Chue-Sang, Yuqiang Bai, Jessica C. Ramella-Roman*



The simultaneous use of multiple imaging modalities can potentially allow for an improved understanding of the results of each individual modality. A combination of Mueller matrix polarimetry and PS-OCT can be used to corroborate results between the different image resolutions, depths of view, and measured polarization-based parameters separately available to the modalities. Birefringence images taken with PS-OCT allow for possible explanations for the changes in the depolarization, diattenuation, and retardation parameters decomposed from a sample's Mueller matrix. It is shown that the decomposed Mueller matrix images show change in areas of abnormal birefringence in heart valve leaflet and tendon caused by injury.



4

3D Interconnectivity of Cortical Tissue Cells

Jared Leichner and Wei-Chiang Lin, PhD*

Macro, Micro and Molecular-scale analyses are essential to understand the complex cortical networks and their alterations in brain tissue. Within the cortex, numerous cell types in the brain interconnect in complex 3D networks to regulate electrical and metabolic activities. By developing a 3D map of cortical tissue interconnectivity that differentiates between various regions of the brain, it will be possible to understand the structural changes that underlie functional differences between brain regions.

Assessing properties of brain tissue in normal and pathological conditions requires an understanding of its hardware (morphological/structural interconnectivity) and software (functional interconnectivity). At this initial stage of examination, only morphological and structural features will be first assessed. Morphological connectivity determines the spacing between cell types in the brain, which affects the speed and magnitude of diffusion of functional molecules. Homogeneous cellular connectivity can help understand the activities of neurons (i.e. action potential propagation, neurotransmitter release) and astrocytes (i.e. calcium wave propagation). Heterogeneous cellular connectivity can help understand tissue-level characteristics, such as neurovascular coupling (neuron-vascular communication) and neurometabolic coupling (neuron-astrocyte communication).

My dissertation work has three fundamental objectives:

1. Determine morphological interconnectivity of neurons, astrocytes and vasculature in the cerebral cortex.
2. Compare connectivity in different regions of cortex.
3. Assess local, regional and global changes in morphology in disease states such as epilepsy

5

Porcine Small Intestinal Submucosal Extra-Cellular Matrix Valve Functionally in the Aortic Position

Omkar Mankame, Makensley Lordeus, Liliam Valdes-Cruz, Steven Bibevski, Frank School, Sarah Bell, Ivan Baez, and Sharan Ramaswamy*

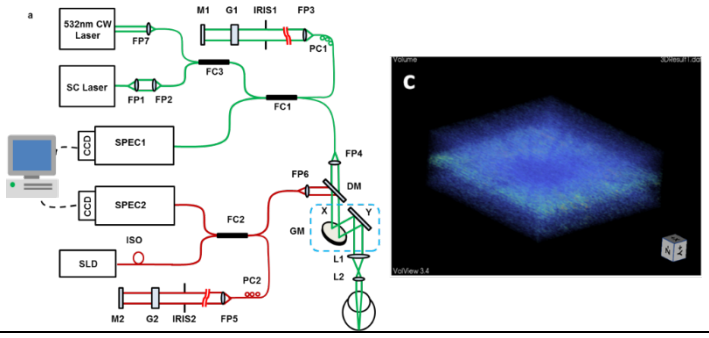
Congenital heart defect is a relatively common complication in our society. One of the more challenging subsets of these patients is young and physically small children presenting with critical heart valve deformities. For these patients, valve replacement procedures are fraught with difficulties and complications since available prosthetic valves have major limitations in terms of growth, potential and longevity. The concept of tissue engineered heart valves (**TEHVs**) which can provide for growth, self-repair, infection resistance, and a permanent approach for replacing defective heart valves is thought to be a potentially ideal solution. Our recent experience suggests that Porcine Small Intestinal Submucosa (PSIS) would be especially attractive for use as a bioscaffold for TEHVs. PSIS material has already been applied by our team for valvular replacement in compassionate care cases involving neonates suffering with critical valve defects. To our knowledge we are the first and only group to have employed this technique for congenital heart valve application. PSIS may possess the ability to recruit endogenous cardiovascular cells, leading to phenotypically matched replacement tissue when the scaffold has completely degraded. As a first step, the aim of this study was to assess the functional effectiveness of PSIS bioscaffolds using a Pulse Duplicator System in our laboratory. It is a pulse simulator which has been modified to evaluate the valves in physiological conditions as that of the functioning heart. Completion of this study will subsequently help to prove our hypothesis of immediate and real utilization of the cell-seeded PSIS-based TEHVs to resemble native valves.

6

Three-dimensional rhodopsin molecular contrast imaging and 3D visualization for functional assessment of photoreceptors

Zahra Nafar, Tan Liu, Rong Wen, Byron Lam, Carmen Puliafit, and Shuliang Jiao*

Rhodopsin, the light-sensing molecule in the outer segments of rod photoreceptors, is responsible for converting light into neuronal signals in a process known as photo-transduction. Rhodopsin is thus a functional biomarker for rod photoreceptors. Here we report a novel technology based on visible light optical coherence tomography (VIS-OCT) for in vivo molecular imaging of rhodopsin. The depth resolution of OCT allows the visualization of the location where the change of optical absorption occurs and provides a potentially accurate assessment of rhodopsin content by segmentation of the image at the location. The technology was successfully tested in vivo by imaging both albino and pigmented rat retina. 3D visualization of rhodopsin in the retina was achieved through speckle suppression by an innovative speckle realignment algorithm. Rhodopsin OCT can be used to quantitatively image rhodopsin distribution and thus assess the distribution of functional rod photoreceptors in the retina. Rhodopsin OCT can bring significant impact into ophthalmic clinics by providing a tool for the diagnosis and severity assessment of a variety of retinal conditions.



7

Histological characterization of IED-generating brain regions using a preclinical model of FCD

Abhay Deshmukh, Jihye Bae, Yinchen Song, and Jorge Riera Diaz*

Current clinical practice of resective surgery in focal epilepsy involves EEG brain source imaging of interictal epileptogenic discharges (IEDs) to localize seizure-onset zones. Unfortunately, recurrent seizures remain after resective surgery in many clinical cases affecting the specificity of this technique. Therefore, a lot of efforts have been made in the past to establish relationships between brain regions associated with IEDs and those underlying ictal epileptogenesis. Limitations of histological studies in human made it attractive to use rat preclinical models to address this issue. Difficulties in the past to perform EEG recording with multiple electrodes in rats have been overcome with a new technology developed in our lab, i.e. the EEG mini-cap. In this study, we propose a methodology to evaluate and validate IED-based localization of epileptogenic brain regions using a rat model of focal epilepsy. To that end, EEG recording with 32 electrodes and histological data were collected from a chronic rat model of focal cortical dysplasia (FCD). This model was created by injecting methylazoxy methanol acetate (MAM) prenatally followed by the I.P administration of pilocarpine to insult the MAM damaged area of the brain, a strategy that induces status epileptius and chronic seizures. IEDs were classified using a method proposed by Bae et al. (2015). Brain source imaging was performed on specific IED subtypes. Anatomical, functional and inflammatory biomarkers were obtained from these IED-generating brain regions and values compared to normal control tissue. We found abnormal anatomical structures in these regions (i.e. larger neuronal processes, glioreactivity and vascular cuffing). We also found increases in expression for ILβ1, TNFα and HMGB1, which are important parameters for inflammation. We conclude that EID-based brain source imaging help localize abnormal tissues highly prospective for epileptogenesis.

8

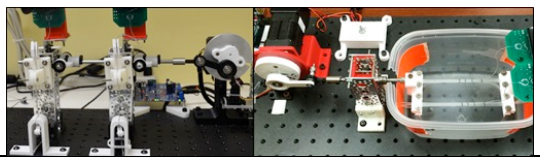
Mechanical fatigue testing of an implantable intrafascicular electrode system

Andres Pena, Sathyakumar Kuntaegowdanahalli, James Abbas, and Ranu Jung*

We have developed an implantable device that uses longitudinal intrafascicular electrodes (LIFEs) to stimulate/record from small groups of fibers in peripheral nerve (PN) fascicles in upper-limb amputees. Since the electrodes and leads must maintain functionality when exposed to stresses during routine activities like walking or lifting, mechanical fatigue testing is necessary to assess the long-term reliability before clinical deployment. The device is comprised of a stimulator/recorder unit with a 15-wire lead assembly that consists of a primary sheathed bundle that leads to a trifurcation junction (TFJ) to form 3 sheathed bundles, each of which further separates into individual LIFEs.

Each LIFE is a 23µm insulated Pt/Ir wire with a 1mm long active zone. Using a needle, each LIFE is sewn longitudinally into the fascicle and sutured to the nerve at the entry and exit points. When implanted, high stresses may occur on the primary bundle near the TFJ, at the point where the individual wires exit the sheath, or at the nerve suture points. Mechanical fatigue at these points could trigger device failure such as breakage of electrode wires or cracks in the sheath or insulation. We have developed equipment and procedures to expose the device to stress conditions that mimic the anticipated stress profiles in the upper arm. One setup imposes bending stress on the lead bundle near the TFJ while it is under tension. Another setup imposes longitudinal strain on LIFE wires anchored to a compliant structure that models the nerve. Electrode continuity was measured periodically and the status of the lead bundles and wires was assessed using a microscope. Two mechanical fatigue test paradigms were used: a high repetition/low amplitude paradigm to mimic activities such as walking (7.3 million cycles based on a 2-year design life at 10,000 steps/day) and a low repetition/high amplitude paradigm to mimic strenuous activities such as lifting (1.2 million cycles; based on OSHA guidelines). Bending amplitudes of ±15° (low) and ±45° (high) were chosen based on ISO standards for a similar device. Strain amplitudes of 5% (low) and 15% (high) were chosen based on nerve strain studies. To-date, all proposed tests have been completed. All wires in all samples have retained electrical continuity and passed visual inspection. These results suggest that this set of leads and fine wires can maintain functionality after deployment in the upper arm

Supported by NIH-R01-EB008578



9

On-Chip SLISA For Rapid Environmental Surveillance of Chemical Toxins

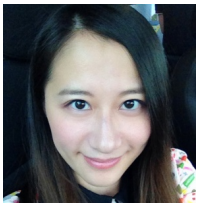
Vinay Bhardwaj, Supriya Srinivasan, and Anthony J. McGoron*

The increasing threat of an intentional (attack) or accidental release of toxins, in particular, chemical-toxins, including chemical warfare agents and toxic industrial chemicals has increased public fear. The major problem in such attacks and accidents is to detect toxins present in very low levels. Indeed, several detection techniques are currently being used for the same. However, none of them meet the most demanding requirements of a detect-to-protect class of biosensors, which is a critical need of federal agencies, including the Department of Homeland Security, Department of Defense and Environmental Protection Agency (EPA).Our group has developed a prototype lab-on-a-chip using silver-based-surface-enhanced Raman spectroscopy (SERS)-Linked ImmunoSensor Assay (SLISA). The on-chip SLISA was tested for the measurement of a stress-marker protein, RAD54, expressed by yeast in response to hydrogen-peroxide, a toxin in the EPA priority list of chemical toxins. This design, for the detection of stress-marker protein in response to toxin (response to dose effect), allows detection of known as well as unknown toxins (global sensing), which can be correlated to human-health using information available on EPA databases. We found, the SLISA has good correlation in accuracy with the standard ELISA technique, and outperforms the latter by being rapid and easy-to-use. SLISA has an edge over ELISA, SLISA is 5 times more sensitive, provides qualitative information on sensor characterization and immunoassay, and allows direct detection with minimal/no chances of uncertainty, a concern in label-based biosensing technologies. The results were correlated to EPA's defined exposure guideline levels of H2O2 to validate the significance of our RISE (rapid, inexpensive, simple and effective) detect-to-protect class of biosensor. The SLISA technique has the potential to be a portable biomedical and environmental sensor technology for primary screening of the biomarkers to toxins, disease and drugs.

10

Wireless- enabled Personal Glucose Meter Coupled with Lateral Flow Immunostrip for Quantitative Detection of Non-glucose Target

Xuena Zhu and Chenzhong Li*



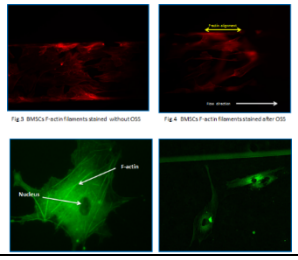
In recent years, much effort has been devoted toward developing point-of-care (POC) devices. Among them, paper-based POC devices is a special category due to the advantages of simple, rapid, on-site, and cost-effective, and has been widely used in home healthcare and medical testing. Lateral flow strip is one of the simplest and most popular formats of paper-based POC devices, and can be used to detect specific substances in a sample by using an immunological reaction. Personal glucose meters (PGMs) is one of the most successfully commercialized diagnostic devices on the market, and it has been widely used by millions of diabetes patients. Generally, glucose meter is capable of detecting glucose as the unique target. To realize non-glucose target detection using a PGM, the relationship between target recognition and glucose generation must be established. Here, we describe a novel design that combines the traditional lateral flow strip with a commercialized PGM for quantitative detection of non-glucose target. The concept was demonstrated by using an oxidative DNA damage biomarker, 8-hydroxy-2'-deoxyguanosine (8-OHdG). The basic design of the device was adapted from our previously reported colorimetric visual detection platform which is based on gold nanoparticles based competitive immunoassay. However, visual detection can provide only qualitative and semi-quantitative results. Thus, to enable quantitative analysis, we establish a novel method that transforms the detection of the target to the detection of an enzyme invertase. The enzyme converts sucrose into glucose for glucose meter readout. The device was able to detect 8-OHdG concentrations in PBS as low as 0.14 ng mL^{-1} with a dynamic range of 0.1-100 ng mL^{-1}. Considering the inherent advantages of the PGM, the demonstration of this device therefore should provide new opportunities for the monitoring of a wide range of biomarkers as well as various target analytes in connection to different molecular recognition events.

11

Bone Marrow Stem Cell Structural Reorganization after Flow Exposure: Relevance to the Valve Phenotype

Glenda Castellanos, L. Nassar, S. Rath, and Sharan Ramaswamy*

Tissue engineered heart valves (TEHV), based on bone marrow stem cells (BMSCs) and biodegradable scaffolds, have been investigated as the next step to current prosthesis limitations particularly lack of somatic growth, which is critical in the pediatric patient population[5]. BMSCs are a promising candidate cell source for tissue heart valve engineering due to its accessibility and phenotypic plasticity under biochemical and mechanical environments. Recent studies have shown that blood shear stress can regulate the proliferation and differentiation of MSCs through a variety of signaling pathways [6]. Heart valves experience mechanical stresses including cyclic flexure, tensile and oscillatory shear stress (OSS) during their lifetime [7]. We previously demonstrated that coupled flexure and flow environments augmented tissue formation using PGA:PLLA scaffolds seeded with BMSCs [8]. Changes in BMSCs' cytoskeleton have also been observed when BMSCs are exposed to OSS. Alterations in F-actin are closely linked to gene expression and protein synthesis in the mechanobiology of stem cells as well as in the differentiation of stem cells. However the pathway by which OSS affects the cell structural response, especially F-actin, of BMSCs is not well understood. Understanding and identifying the mechanisms by which cytoskeletal changes may lead to cellular differentiation of a valvular phenotype is a first critical step in enhancing the promotion of a robust valvular phenotype from BMSCs.



12

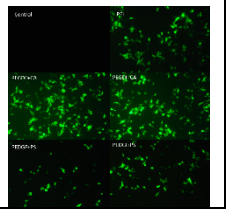
Modified PEGDF and PEGDI Polymers for Non-Viral Gene Delivery in HEK 293 Cells

Anh Le

Gene therapy involves the use of nucleic acids, either DNA or RNA for the treatment, cure, or prevention of human diseases. Synthetic cationic polymers are promising as a tool for gene delivery because of their high level of design flexibility for biomaterial construction and are capable of binding and condensing DNA through electrostatic interactions.

Our lab have developed a novel polymer (poly (polyethylene glycol-dodecanoate) (PEGD), a polyester of polyethylene glycol (PEG) and dodecanedioic acid (DDA). PEGD is a linear viscous polymer that self-assembles into a vesicle upon immersion in an aqueous solution. A copolymer of dodecanedioc acid and polyethylene glycol (PEG) was synthesized at a 1:1 ratio. Furmaric (FA) or itaconic acid (IA) was used to suppress DDA in the PEGD copolymer at an 80:20 ratio (DDA: furmaric/itaconic acid) to form the PEGDF/I variant. PEGDF/I are then modified through the Michael addition of Protamine Sulfate (PEGDF/I-PS) and Cys-Arg_8 (PEGDF/I-CA) peptide to the carbon-carbon double bond on the polymer backbone to introduce a positive charge.

The modified PEGDF/I polymers were capable of binding and condensing DNA. Transfection of HEK 293 cells with pTurboGFP plasmid using modified PEGDF/I polymers were successful and showed varied efficiency. Cell viability testing via Alamar Blue Assay showed that PEGDF-PS and PEGDI-PS were toxic when compared to the control, whereas PEGDF-CA and PEGDI-CA were non-toxic. PEGDF/I-CA polymer were more efficient at transfecting HEK293 cells than their had around 30% transfection efficiency



13

Adaptive control of lung volume for respiratory pacing in the rodent model

Ricardo Siu, Brian Hillen, Anil Thota, James Abbas, Sylvie Renaud, and Ranu Jung*

High-cervical spinal cord injury can lead to respiratory deficiency due to paralysis of inspiratory muscles. Functional electrical stimulation (FES) has been applied to restore ventilatory function in individuals with respiratory deficiency as an alternative to mechanical ventilation. Control paradigms for FES are often based on open-loop controllers that depend on careful calibration and setup by a clinician and technician but are unable to adapt to a patient's respiratory demand and changes in electrode properties. Our goal is to develop a neuromorphic controller that can adapt to the respiratory needs of the user based on physiological feedback values.

A software-based adaptive controller was implemented to modulate the amplitude of stimulation pulses delivered to the diaphragm based on lung volume feedback in an uninjured, anesthetized rat model. Stimulation pulse width (200 µs) and frequency (75 Hz) were held constant. Lung volume was derived through real-time integration of the flow signal from a pneumotachometer incorporated into the breathing circuit. Native breath volume was used to determine a baseline breath volume target. To assess the ability of the system to adjust ventilation in a controlled manner, the targeted lung volume trajectory was scaled to 120% of the baseline breath volume to mimic the response to hypercapnia observed in previous studies with anesthetized rodents; respiratory period was kept at a constant value determined by initial breathing conditions. The controller achieved an adequate and steady breathing pattern within 25 - 30 cycles after initiating stimulation at baseline target. After increasing the target volume to 120% of baseline, the controller adapted to the change in 10 cycles. After reducing the desired volume back to baseline, the controller achieved adaptation in 15 cycles. These results demonstrate the ability of the adaptive controller to automatically achieve a specified ventilatory pattern.

14

Portable Colposcope Towards the Prediction of Preterm Birth Using Full Mueller Matrix Imaging of Cervical Collagen

Susan Stoff, Nola Holness, Amir Gandjbakhche, Vikto Chernomordik, and Jessica Ramella-Roman*

Although preterm birth is the number one cause of infant mortality and neurological disorders in the world, there is not, yet, a reliable or accurate method for diagnosing women at risk of preterm labor. Cervical collagen is the main component for providing strength and maintaining the weight and structure of the cervix in order for the fetus to gestate. As pregnancy progresses, cervical collagen becomes more disorganized and allows the weakening and opening of the cervix for birth. The changes in cervical collagen may occur prematurely in preterm birth. These changes in collagen organization can be analyzed using Mueller Matrix Polarimetric imaging of the characteristic birefringence of collagen. In this research, we have built a full Mueller Matrix Polarimetry attachment to a standard colposcope to enable imaging and analysis of the quantity and organization of cervical collagen throughout pregnancy.

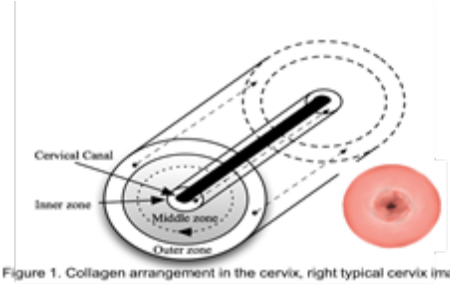


Figure 1. Collagen arrangement in the cervix, right typical cervix image

15

NIROS for non-contact hemodynamic imaging: Instrument development

Arash Dadkhah, Jiali Lei, and Anuradha Godavarty*

A near-infrared optical scanner (NIROS) has been developed for non-contact sub-surface imaging of wounds at Optical Imaging Laboratory (OIL). Preliminary studies revealed that the NIR optical contrast can differentiate healing from non-healing wounds. There is a need to understand the changes in the blood flow in terms of changes in oxy-(HbO) and deoxy- hemoglobin (HbR) at the wound site, apart from the optical changes. The current device, NIROS employs two LED light sources of single wavelength (710 nm and 830 nm) to illuminate tissue surface during diabetic foot imaging studies. Most of the time, the overlap between two LED sources was unstable and minimal resulting in disruption of accurate signal extraction for HbO and HbR. Moreover, it is unable to uniformly illuminate the region of interest. Therefore, the objective of the research is to modify the NIROS such that it can perform real-time hemodynamic imaging in-vivo on normal foot (and in diabetic foot ulcers in the future). To do this, the first step is to modify the source system of NIROS to allow simultaneous imaging at multi NIR wavelengths to reach maximum possible uniform illumination area, towards hemodynamic imaging. Herein, the source system of NIROS was modified and changed to single multi-wavelength LED and incorporated with diffuser, such that the area of illumination uniformly overlaps to the maximum area possible to assess the changes in blood flow, in terms of changes in HbO and HbR.

Keywords: Near-infrared optical scanner (NIROS); Diabetic foot ulcers; oxy-(HbO) and deoxy- hemoglobin (HbR); Multi-wavelength LED;