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TITLE: Noninvasive Optical Monitoring of Spinal Cord Hemodynamics and Oxygenation after Acute Spinal Cord Injury

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Our objectives in Year 2 are fully addressed. Following a series of technical refinements and pilot studies. OXT5 (V1) NIRS sensor prototype and our Multi-Wavelength NIRS system were developed. This setup was required for monitoring spinal cord oxygenation, hemodynamics and cytochrome c oxidase (CCO) activity in our animal model of acute SCI (Experiment 1). We successfully completed Experiment 1 as scheduled in year 2. Nine animals were studied and comparative statistical analysis was completed. Outcomes of Experiment 1 are prepared for publication in the Journal of Neurotrauma. We will also present our results at 2018 Neuroscience conference in San Diego and at 2019 SPIE Photonics West conference in San Francisco. Following completion of Experiment 1, we proceed by conducting the 3rd round of NIRS technology development to design and prototype miniaturized V2-NIRS sensor and modified NIRS system enabled for continuous long-term data collection. V2-NIRS sensor is being tested in a series of pilot animal studies. In year three we conduct Experiment 2 and will focus on refining the technology to engineer a clinical spinal cord NIRS sensor.

15. SUBJECT TERMS

Spinal Cord Injury, Hemodynamic Support, Spinal Cord hemodynamics, Spinal Cord Blood Flow, Near Infrared Spectroscopy, Intraparenchymal Pressure

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1 INTRODUCTION

The hemodynamic management of acute spinal cord injury (SCI) represents an under-appreciated opportunity to improve neurologic recovery in human patients. A major limitation in our ability to optimize hemodynamic management in acute SCI is the lack of a real-time method for measuring blood flow, oxygenation, metabolic responses, and hydrostatic pressure within the injured spinal cord. Near-infrared spectroscopy (NIRS) offers the potential to provide a relatively non-invasive measure of these important parameters within the injured spinal cord. NIRS works by transmitting near infrared light through tissue, and based on the absorption of this light by chromophores such as oxygenated and deoxygenated hemoglobin (O_2Hb and HHb), microcirculatory oxygen and perfusion can be derived. Additionally, alterations in the O_2Hb waveform that are caused by tissue pressure can be potentially utilized to monitor changes in hydrostatic pressure within the cord. Finally, NIRS measures of the redox state of cytochrome-c-oxidase (CCO) can provide information not just about tissue O_2 but also about downstream cellular O_2 metabolism. The overall objective of this initiative is to develop an implantable NIRS sensor and system that can be used to provide non-invasive real-time measurements of spinal cord oxygenation, blood flow, pressure, and oxidative metabolism in acute human SCI. We will test the hypothesis that a NIRS sensor positioned extra-durally can provide real-time measurements of tissue oxygenation, perfusion, oxidative metabolism and hydrostatic pressure within the underlying spinal cord adjacent to the site of traumatic injury over the course of seven-day post-injury. To test this hypothesis, we will conduct a series of preclinical studies using our pig model of thoracic SCI, alongside efforts to refine the NIRS technology into a clinically applicable device. Our animal studies aim to establish the relationship between non-invasive NIRS measurements of oxygenation, perfusion, metabolism and pressure with invasive IP monitoring that is made possible by the large calibre of the pig spinal cord. First, we will conduct a non-survival study in 8 animals to verify the relationship between NIRS and IP spinal cord monitoring after various intra-operative stimuli, including systemic hypoxia, contusive SCI, sustained spinal cord compression, and alterations in blood pressure. After refining the technology to engineer a sensor that can potentially be used in humans, we will test the NIRS system in another pig study with a seven-day post-injury survival period to determine how well the NIRS system monitors tissue changes in comparison to Intraparenchymal (IP) monitoring in awake, mobile animals (a more clinically relevant scenario).

2 KEYWORDS

- Spinal Cord Injury
- Hemodynamic Support
- Spinal Cord hemodynamics
- Spinal Cord Blood Flow
- Near Infrared Spectroscopy
- Intraparenchymal Pressure

3 ACCOMPLISHEMENTS

3.1 Protocol and Activity Status

- **Human Use Regulatory Protocols**
  No human subject research will be performed to complete the Statement of Work

- **Use of Human Cadavers for RDT&E, Education or Training**
  No RDT&E, education or training activities involving human cadavers will be performed to complete the Statement of Work

- **Animal Use Regulatory Protocols**

  Total Protocols: Two animal use research protocol will be required to complete the Statement of Work

- **Protocol: 1 of 1**
- **Protocol [ACURO Assigned Number]:** Conveyed from SC130007 and SC130008
- **Title:** Optical Monitoring of Spinal Cord Hemodynamics
- **Target required for statistical significance**: n=8 / each of two experiments
- **Target approved for statistical significance**: n=8 / each of two experiments
- **Submitted to and Approved by:** Bryan K. Ketzenberger, DVM, DACLAM
- **Status:** Approved - August 15, 2016

3.2 Approved Statement of Work

The approved statement of work is described below. A current Gantt chart is provided in Table 1 for reference.

Table 1: Approved Statement of Work (Gantt chart).

Specific Aim 1: *Refine our current NIRS sensor (V1) configuration, and software algorithms to optimize the measurement of spinal cord oxygenation (O_2Hb, HHb, TOI%), perfusion (THb), metabolism (CCO) and hydrostatic pressure.*	**YEAR 1**				**YEAR 2**				**YEAR 3**			
Activity	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
1a) UBC & ACURO approvals												
1b) Technology assessment												

1c) IP assessment												
1d) V1 Sensor development												
Specific Aim 2: *NIRS system evaluation and verification of the relationship between the NIRS and IP assessments of oxygenation, perfusion, pressure, and metabolic responses in our pig SCI model in anesthetized (n=8 immobile) animals (Experiment 1)*	**YEAR 1**				**YEAR 2**				**YEAR 3**			
Activity	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
2a) NIRS system evaluation and collecting NIRS and IP data in Experiment 1												
2b) Data analysis: NIRS vs. IP measurements (SC oxygenation & perfusion)												
2c) Data analysis: NIRS vs. IP measurements (SC pressure)												
2d) Data analysis: NIRS vs. IP measurements (SC metabolic responses)												
2e) Final data analysis and dissemination												
Specific Aim 3: *Refine and miniaturize the NIRS sensor to develop an implantable (but also removable), NIRS sensor (V2) that can be used to monitor the spinal cord for a clinically relevant time frame post-injury in the "non-ideal" setting of an awake (and moving) individual.*	**YEAR 1**				**YEAR 2**				**YEAR 3**			
Activity	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
3a) V2 sensor development												
3b) V2 sensor calibration												
3c) NIRS system refinement for long term (7-day) monitoring												

Specific Aim 4: *Establish the ability of V2 sensor to assess oxygenation, perfusion, pressure, and metabolic responses for 7-day post-injury by comparing the NIRS and IP measurements in pigs that are awake and mobile for 7 days post-injury. (n=8)*	**YEAR 1**				**YEAR 2**				**YEAR 3**			
Activity	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
4a) NIRS vs. IP measurements (SC oxygenation & perfusion)												
4b) NIRS vs. IP measurements (SC pressure)												
4c) NIRS vs. IP measurements (SC metabolic responses)												
4d) Data analysis, dissemination												
Specific Aim 5: *Obtain local ethics approval to conduct a clinical trial of our finalized NIRS system in human acute SCI patients.*	**YEAR 1**				**YEAR 2**				**YEAR 3**			
Activity	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
5a) Final refinements of V3 sensor												
5b) Obtaining Canada Health approval for the V3 sensor												
5c) Obtaining UBC CREB approval												

Aim 1. Refine our current NIRS sensor (V1) configuration, and software algorithms to optimize the measurement of spinal cord (SC) oxygenation (O_2Hb, HHb, TOI%), perfusion (THb), metabolism (CCO) and hydrostatic pressure.

Task 1: Submit documents for Institutional (UBC) and ACURO approval. [Months 1-3]
Task 2: NIRS technology assessment. [Months 1-6]
Task 3: Intellectual Property (IP) protection assessment, through UBC University Industrial Liaison office (UILO). [Months 1-6]
Task 4: Develop SC NIRS sensor V1 to be used in experiment 1. [Months 5-9]

Milestone(s) Achieved:

(a) *UBC and ACURO approvals are obtained.*

(b) *NIRS technology assessment is completed.*

(c) *The first round of the Invention Disclosure assessment is completed by the UBC University Industry Liaison Office (UILO); file number: 17-037.*

(d) *The first SC-NIRS sensor (V1) was developed, modified and examined in a pilot study on five pig models of SCI during March 13 - May 8, 2017. The final version of the V1 NIRS sensor was prototyped through the middle of May and is now is being used in Experiment 1.*

(e) *After extensive consultations with various NIRS manufacturers, such as Hamamatsu during technology assessment stage (Task 2), we came to the conclusion that currently available NIRS technology and equipment do not have the capacity to reliably and accurately monitor changes of spinal cord cytochrome c oxidase (COO), at least in a manner that would be applicable to eventual clinical application. Almost all researchers conducting CCO measurements are using experimental lab-based systems that are not applicable in clinical settings. To approach this challenge, we worked with a biophotonics consulting company in January 2017 to design and prototype a unique NIRS system that may enable us to detect spinal cord CCO changes. Pathonix Innovation Inc. is a Canadian biophotonics technology consulting company that has been actively in the field of design and customizing NIRS systems applied by the clinical researcher in different universities and institutions including US National Institute of Health (NIH). After consultations and reviewing the task with Pathonix engineers, a novel multi-wavelength NIRS prototype for monitoring changes of tissue CCO as well as O_2Hb and HHb was developed. Following a series of sensor and software refinements, the new MW-NIRS sensor (OXT5) could successfully collect CCO traces in three pilot animal models of acute SCI. The final waterproof and advanced version of OXT5 prototype, was used to collect all NIRS traces in Experiment 1.*

(f) *The protocol of Experiment 1 was tested and refined.*

Aim 2. Verify the relationship between the NIRS and intraparenchymal (IP) assessments of oxygenation, perfusion, pressure, and metabolic responses in our pig SCI model in anesthetized (immobile) animals.

Task 1: Evaluating the NIRS system function in a pig model of SCI in conjunction with IP measurements of oxygen, perfusion, pressure, and metabolism. (Non-survival experiment with NIRS & IP monitoring under anesthesia, n=8)

Task 2: Comparing tissue oxygenation & perfusion as assessed by 1) NIRS measures of O_2Hb, HHb, THb, TOI% and Hbdiff, and 2) an IP oxygen/blood flow sensor. [Months 10-14]

Task 3: Comparing hydrostatic pressure as assessed by 1) a novel analysis of the NIRS O_2Hb waveform that accounts for how cardiac-induced oscillations in the waveform are influenced by tissue pressure, and 2) an IP pressure sensor. [Months 10-14]

Task 4: Comparing metabolic responses as assessed by 1) NIRS measures of cytochrome-c-oxidase (CCO), and 2) an IP microdialysis catheter to monitor lactate, pyruvate, and the lactate/pyruvate ratio. [Months 10-14]

Task 5: Data analysis. [Month 15]

Milestone(s) Achieved:

(a) *Experiment 1 is completed. Spinal cord NIRS data were successfully collected by OXT5 NIRS system, in conjunction with IP measurements of spinal cord tissue oxygenation, perfusion, pressure, and metabolism. NIRS sensor placement and on-going monitoring were completed in all animals and were not associated with any complications.*

(b) *Comparisons of NIRS measures of spinal cord oxygenation and hemodynamics with IP measures of spinal cord oxygenation and blood flow were completed.*

(C) *Comparison of NIRS measure of spinal cord CCO activity with IP measure of spinal cord metabolism, L/P ratio, was completed.*

(d) *Data analysis of Experiment 1 is completed.*

Aim 3. Refine and miniaturize the NIRS sensor, cables, and fixation devices to develop an implantable (but also removable), NIRS sensor (V2) that can be used to monitor the spinal cord for a clinically relevant time frame post-injury in the "non-ideal" setting of an awake (and moving) individual.

Task 1: NIRS monitoring system adjustment/refinement for long term (7-day) continuous monitoring, data storage and management. [Months 16-20]

Task 2: Sensor prototyping and development. [Months 16-19]

Task 3: Sensor calibration and testing. [Months 19-20]

Milestone(s) Achieved:

(a) *A new miniaturized version of the OXT5 sensor, named V2-NIRS sensor, is developed by our technology developer partner. We also upgraded our NIRS hardware, firmware and operating software. The new V2-NIRS sensor is a miniaturized version of OXT5 NIRS sensor with a longer and special unified cable (2.5 m), that includes eight shielded wires, to better protect against potential Electromagnetic interference (EMI). The diameter of the cable and the optode is 6 mm. A copy of the system development proposal is attached (Attachment 1).*

(b) *To provide stable and consistent contact between V2-NIRS sensor optode and the spinal cord tissue during data collection, we have designed an adjustable magnetic fixator, with three degrees of freedom.*

(c) *We are currently testing V2-NIRS sensor function, modified NIRS hardware and the NIRS magnetic fixator system in a series of pilot animal models of acute (#2) and survival (#2) SCI.*

Aim 4. Establish the ability of this refined NIRS system to assess oxygenation, perfusion, pressure, and metabolic responses for 7-day post-injury by comparing the NIRS and IP measurements in pigs that are awake and mobile in their cages for 7 days post-injury.

Task 1: Comparing changes in tissue oxygenation & perfusion as assessed by 1) NIRS measures of O_2Hb, HHb, THb, and TOI%, and 2) an IP oxygen/blood flow sensor. [Months 21-28]

Task 2: Comparing changes in hydrostatic pressure as assessed by 1) a novel analysis of the NIRS O_2Hb waveform that accounts for how cardiac-induced oscillations in the waveform are influenced by tissue pressure, and 2) an IP pressure sensor. [Months 21-28]

Task 3: Comparing changes in metabolic responses as assessed by 1) NIRS measures of cytochrome-c-oxidase (CCO), and 2) an IP microdialysis catheter to monitor lactate, pyruvate, and the lactate/pyruvate ratio. [Months 21-28]

Task 4: Data analysis and dissemination. [Month 29]

Milestone(s) Achieved:
Nothing to report

Aim 5. Obtain local ethics approval to conduct a clinical trial of our finalized NIRS system in human acute SCI patients.

Task 1: Final refinements and development of a clinical sensor (V3) for a future pilot trial in human. [Months 30-34]

Task 2: Obtaining Canada Health Approval for the V3 sensor. [Months 32-36]

Task 3: Obtaining UBC CREB approval for conducting a clinical trial in acute SCI patient to test the V3 feasibility and safety. [Months 32-36]

Milestone(s) Achieved:
Nothing to report

3.3 Current Progress on Statement of Work

A Gantt chart indicating actual completed works is provided in Table 2 for reference.

Aim 1. Refine our current NIRS sensor (V1) configuration, and software algorithms to optimize the measurement of spinal cord (SC) oxygenation (O_2Hb, HHb, TOI%), perfusion (THb), metabolism (CCO) and hydrostatic pressure.

- **Task 1:** Submit documents for Institutional (UBC) and ACURO approval.

  Completed. *We have obtained UBC and ACURO approval. This protocol was approved by the University of British Columbia, Vancouver IACUC on January 31, 2016. Dated August 15, 2016, ACURO approval was conveyed to project protocol from USAMRMC protocols SC130007 and SC130008 which were previously approved for the use of swine.*

- **Task 2:** NIRS technology assessment.

  Completed. *An updated literature review on the technology applied in this project is completed. The NIRS technologies and engineering components required for the*

development of the first (V1) sensor prototype is defined. The first series of equipment related to V1 sensor development and Experiment 1 are provided.

- **Task 3:** Intellectual Property (IP) protection assessment, through UBC University Industrial Liaison office (UILO).

  In progress. *Our intellectual property application was reviewed by the UBC UILO (file#: 17-037). We are planning to file an IP related to our MW-NIRS method in Year 3.*

- **Task 4:** Develop SC-NIRS sensor V1 to be used in Experiment 1.

  Completed.

Aim 2. Verify the relationship between the NIRS and intraparenchymal (IP) assessments of oxygenation, perfusion, pressure, and metabolic responses in our pig SCI model in anesthetized (immobile) animals.

- **Task 1:** Evaluating the NIRS system function in a pig model of SCI in conjunction with IP measurements of oxygen, perfusion, pressure, and metabolism.

  Completed.

- **Task 2:** Comparing tissue oxygenation & perfusion as assessed by 1) NIRS measures of O_2Hb, HHb, THb, and TOI%, and 2) an IP oxygen/blood flow sensor.

  Completed.

- **Task 3:** Comparing hydrostatic pressure as assessed by 1) a novel analysis of the NIRS O_2Hb waveform that accounts for how cardiac-induced oscillations in the waveform are influenced by tissue pressure, and 2) an IP pressure sensor.

  In progress. *Data analysis and comparisons are in progress. We are hiring a signal processing expert to optimize NIRS waveform analysis method that is required for noninvasive tissue hydrostatic pressure monitoring.*

- **Task 4:** Comparing metabolic responses as assessed by 1) NIRS measures of cytochrome-c-oxidase (CCO), and 2) an IP microdialysis catheter to monitor lactate, pyruvate, and the lactate/pyruvate ratio.

  Completed.

- **Task 5:** Final data analysis and dissemination.

Completed. *Data analysis.*

In progress. *A manuscript is prepared for submission to the Journal of Neurotrauma in October. Results of Experiment 1 will be presented at 2018 SFN conference in San Diego and at 2019 SPIE Photonics West in San Francisco.*

Aim 3. Refine and miniaturize the NIRS sensor, cables, and fixation devices to develop an implantable (but also removable), NIRS sensor (V2) that can be used to monitor the spinal cord for a clinically relevant time frame post-injury in the "non-ideal" setting of an awake (and moving) individual.

- **Task 1:** NIRS system adjustment/refinement for long term (7-day) continuous monitoring of the spinal cord, data storage and management.

  Completed.

- **Task 2:** Sensor prototyping and development.

  Completed.

- **Task 3:** Sensor calibration and testing.

  In progress. *V2-NIRS prototype and system is being tested in a series of pilot animal studies.*

Aim 4. Establish the ability of this refined NIRS system to assess oxygenation, perfusion, pressure, and metabolic responses for 7-day post-injury by comparing the NIRS and IP measurements in pigs that are awake and mobile in their cages for 7 days post-injury.

- **Task 1:** Comparing changes in tissue oxygenation & perfusion as assessed by 1) NIRS measures of O_2Hb, HHb, THb, and TOI%, and 2) an IP oxygen/blood flow sensor.

  Nothing to report

- **Task 2:** Comparing changes in hydrostatic pressure as assessed by 1) a novel analysis of the NIRS O_2Hb waveform that accounts for how cardiac-induced oscillations in the waveform are influenced by tissue pressure, and 2) an IP pressure sensor.

  Nothing to report

- **Task 3:** Comparing changes in metabolic responses as assessed by 1) NIRS measures of cytochrome-c-oxidase (CCO), and 2) an IP microdialysis catheter to monitor lactate, pyruvate, and the lactate/pyruvate ratio.

  Nothing to report

- ***Task 4:*** *Data analysis and dissemination.*

  Nothing to report

Aim 5. Obtain local ethics approval to conduct a clinical trial of our finalized NIRS system in human acute SCI patients.

- **Task 1:** Final refinements to develop a clinical sensor (V3) for a future pilot trial in human.

  Nothing to report

- **Task 2:** Obtaining Canada Health Approval for the V3 sensor.

  Nothing to report

- **Task 3:** Obtaining UBC CREB approval for conducting a clinical trial in acute SCI patient to test the V3 feasibility and safety.

  Nothing to report

Table 2: Gantt chart of current work. Blue sections reflect actual work completed. Green sections indicate in progress tasks. This Gantt chart of current work matches the Gantt chart of approved statement of work (Table 1).

Specific Aim 1: *Refine our current NIRS sensor (V1) configuration, and software algorithms to optimize the measurement of spinal cord oxygenation (O_2Hb, HHb, TOI%), perfusion (THb), metabolism (CCO) and hydrostatic pressure.*	**YEAR 1**				**YEAR 2**				**YEAR 3**			
Activity	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**

1a) UBC & ACURO approvals	■											
1b) Technology assessment	■	■										
1c) IP assessment	■	■										
1d) V1 Sensor development		■	■									
Specific Aim 2: *NIRS system evaluation and verification of the relationship between the NIRS and IP assessments of oxygenation, perfusion, pressure, and metabolic responses in our pig SCI model in anesthetized (n=8 immobile) animals (Experiment 1)*	**YEAR 1**				**YEAR 2**				**YEAR 3**			
Activity	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
2a) NIRS system evaluation and collecting NIRS and IP data in Experiment 1				■	■							
2b) Data analysis: NIRS vs. IP measurements (SC oxygenation & perfusion)				■	■							
2c) Data analysis: NIRS vs. IP measurements (SC pressure)				■	■							
2d) Data analysis: NIRS vs. IP measurements (SC metabolic responses)				■	■							
2e) Final data analysis and dissemination					■							
Specific Aim 3: *Refine and miniaturize the NIRS sensor to develop an implantable (but also removable), NIRS sensor (V2) that can be used to monitor the spinal cord for a clinically relevant time frame post-injury in the "non-ideal" setting of an awake (and moving) individual.*	**YEAR 1**				**YEAR 2**				**YEAR 3**			
Activity	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
3a) V2 sensor development						■	■					

3b) V2 sensor calibration												
3c) NIRS system refinement for long term (7-day) monitoring												
Specific Aim 4: *Establish the ability of V2 sensor to assess oxygenation, perfusion, pressure, and metabolic responses for 7-day post-injury by comparing the NIRS and IP measurements in pigs that are awake and mobile for 7 days post-injury. (n=8)*	**YEAR 1**				**YEAR 2**				**YEAR 3**			
Activity	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
4a) NIRS vs. IP measurements (SC oxygenation & perfusion)												
4b) NIRS vs. IP measurements (SC pressure)												
4c) NIRS vs. IP measurements (SC metabolic responses)												
4d) Data analysis, dissemination												
Specific Aim 5: *Obtain local ethics approval to conduct a clinical trial of our finalized NIRS system in human acute SCI patients.*	**YEAR 1**				**YEAR 2**				**YEAR 3**			
Activity	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**	**Q1**	**Q2**	**Q3**	**Q4**
5a) Final refinements of V3 sensor												
5b) Obtaining Canada Health approval for the V3 sensor												
5c) Obtaining UBC CREB approval												

4 OVERALL PROJECT SUMMARY

The overall objective of this project is to develop a NIRS sensor, system and method that can provide real-time monitoring of spinal cord oxygenation, blood flow, pressure, and metabolic responses after acute SCI. The ability to monitor these parameters within the

injured spinal cord will provide clinicians with potentially critical information to optimize the hemodynamic management of the acutely injured patient. In this grant, we propose a sequence of preclinical studies aimed to translate this approach to human SCI patients. Following initial NIRS technology assessmsnt, prototype design and development, and a series of pilot animal studies and technology refinements in year one, Experiment 1 was completed in year two.

EXPERIMENT 1

4.1 METHODS

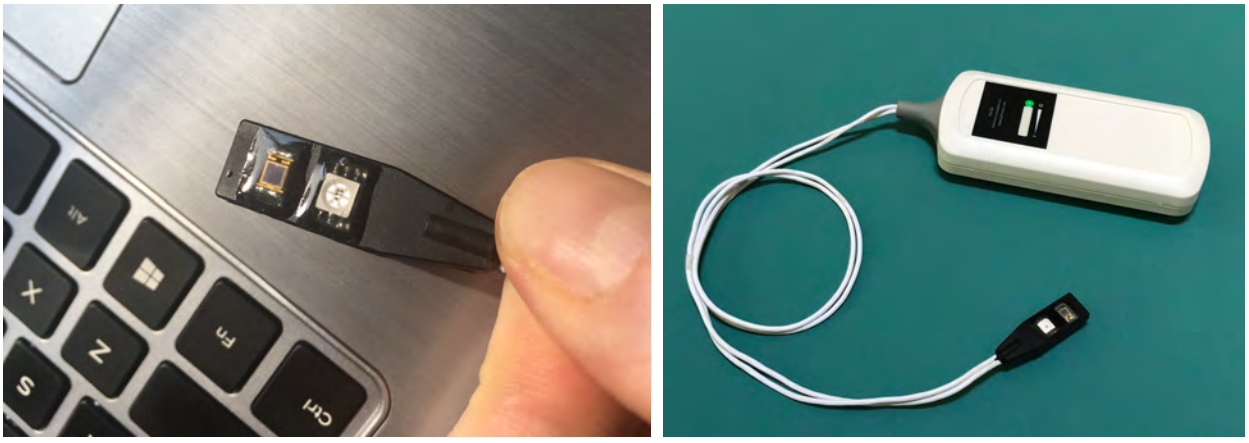
4.1.a NIRS Prototype Development

OXT5 NIRS – The custom NIRS sensor that was developed for our study (OXT5) employs a miniaturized optode (4 x 10 x 20 mm), where one single multi-wavelength (MW) surface-mount-device light-emitting diode (SMD-LED) with five wavelengths (660, 730, 810, 850, 950 nm), and an output power of 2 mW, is linked to a single photodetector (Figure 1). They are configured to give an inter-optode distances (IOD) of 10 mm.

Figure 1. First OXT5 prototype

After a trial we realized that the sensor needs to be completely fluid resistant while being fully transparent. The second version of the OXT5 was therefore covered by an ultra clear silicon shield to protect it from blood and other fluids while optimizing light transmission (Figure 2). The sensor was designed to be small enough to rest directly on the dural surface of the spinal cord, while connected by a flexible biocompatible multi-branch shielded wire to a compact MW-NIRS system that was placed outside the animal. The NIRS system used a continuous-wave time-multiplexing configuration, with a mathematical algorithm to translate light attenuation for each wavelength absorbed by O_2Hb and HHb chromophores to their relative concentration changes.

Figure 2. Modified version of OXT5 sensor.



The resulting relative concentration changes are the product of scaling the absolute concentration changes by an undetermined scaling factor. This factor, which is called the differential pathlength, is the average distance a photon travels between the source and detector through the tissue. The extinction coefficients were referenced from the literature. By increasing the number of wavelengths (i.e. to more than the conventional two wavelengths), and solving for multiple optical density equations at each wavelength, our MW-NIRS system provides more accurate measurement of tissue O_2Hb and HHb as well as measuring CCO chromophore changes. The algorithm used is derived from the modified Beer-Lambert Law; THb and Hb difference [Hbdiff = (O_2Hb) – (HHb)] are also calculated. Hbdiff is a NIRS parameter of tissue oxygenation. The NIRS system also calculates the TOI%, which is an absolute measure of tissue oxygenation status. This is done by a second algorithm which uses the light attenuation of the five wavelengths. A laptop computer equipped with custom software collects and records NIRS signals at 100 Hz, and analyzes and displays tissue O_2Hb, HHb, CCO, THb, Hbdiff and TOI% graphically in real time.

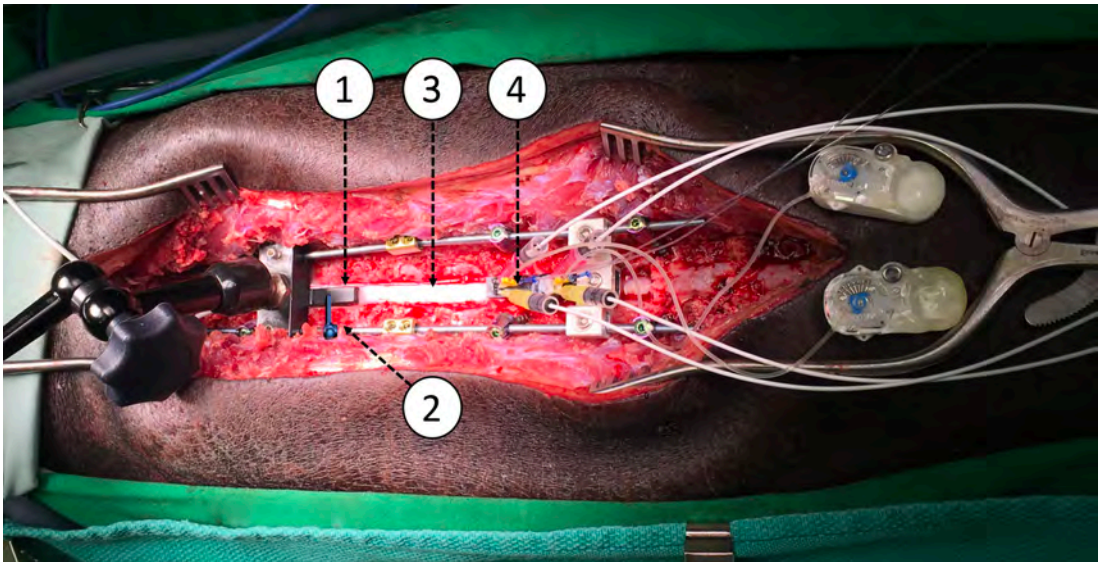
4.1.b Animal and Surgical Preparation

Animals were prepared for surgery as previously described.[1] Arterial oxygen saturation (SaO_2) and heart rate (HR) pulsation were monitored with a pulse oximeter (8600 V, Nonin Medical, MN) attached to the animal's ear. Ventilation was set at 17 breaths per minute and the animal was initially oxygenated with a combination of 1.4L (70%) nitrogen and 0.6L (30%) oxygen. The left jugular vein and carotid artery were exposed via blunt dissection. A 20-gauge catheter was inserted into the carotid artery and connected to a fluid filled pressure transducer to yield invasive MAP throughout the experiment. The jugular vein was catheterized for infusion of norepinephrine (NE) and nitroprusside (NP)

to purposefully manipulate MAP. The right femoral artery and vein were also exposed and cannulated for collecting arterial and blood samples for blood gas (BG) analysis.

Following surgical preparation, the animal was a positioned into a prone position. The posterior spine was then surgically exposed between the T4-L3 spine levels. The T9, T10, and T11 pedicles were cannulated and instrumented with 3.5 x 25 mm polyaxial screws (Vertex screws, Medtronic, Memphis, TN). Then a laminectomy was carried out at the T5 to L1 levels to expose the dura and spinal cord. Prior to SCI, a total of three IP probes were inserted through the dura into the spinal cord at T11 for the assessment of blood flow/partial pressure of oxygen (NX-BF/OF/E, Oxford Optronix, Oxford, UK), hydrostatic pressure (FOP-LS-NS-1006A, FISO Technologies Inc., Harvard Apparatus, Quebec, Canada), and microdialysis (CMA11, CMA Microdialysis, Harvard Apparatus, Quebec, Canada). IP probes were advanced to a depth of 4 mm, approximately 2 cm caudal from the anticipated edge of the impactor tip location.[1] Probes were inserted using custom made cannulas positioned in a sensor holder secured to the spine via 3.5 mm titanium rods (Medtronic), and pedicle screws (Medtronic) at T9, T11, T12 and T14. 23 Accurate positioning of the probes into the cord was verified by Ultrasound imaging (L14-5/38, 38mm linear array probe, Ultrasonix RP; BK Ultrasound, Richmond, British Columbia). After placement of IP catheters, the NIRS sensor was placed and fixed on top of the dura at the T9 level and secured with a cross connector. The NIRS sensor was lowered directly onto the surgically-exposed dura (Figure 3). The wires of the sensor and all probes were brought out of the surgical field, fixed over the back of the animal and connected to their respective monitoring units.

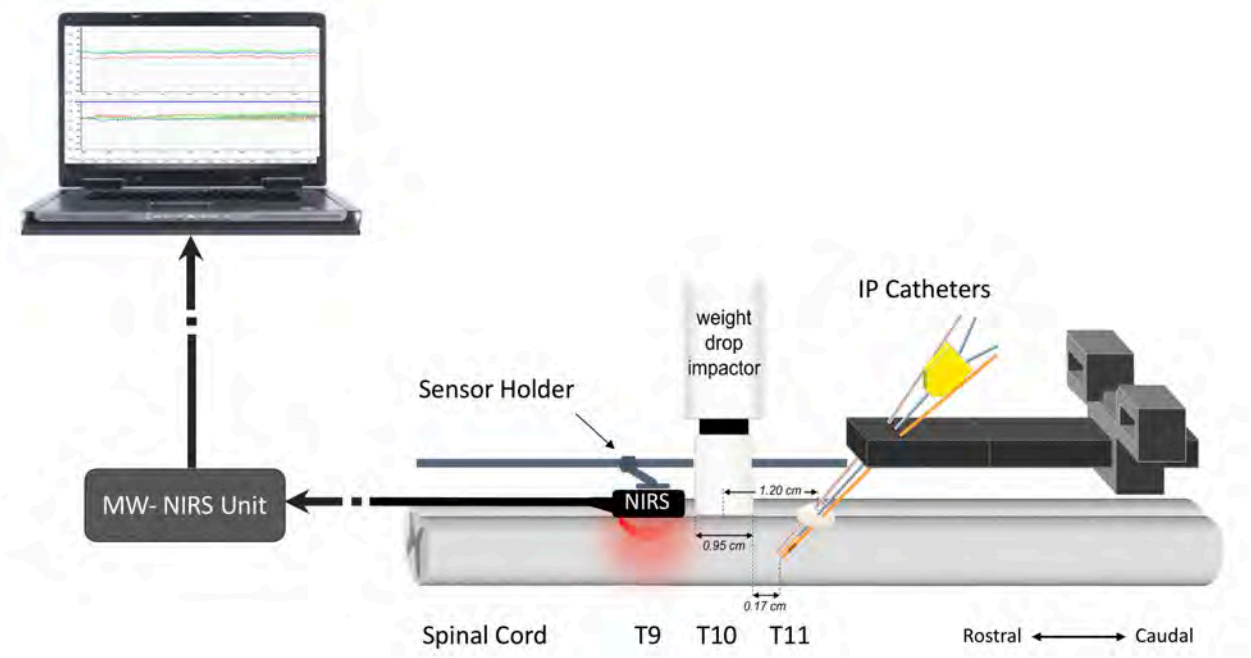
Figure 3. The custom-made OXT5 sensor (1) placed and fixed on the spinal cord (3) by a fixator (2), and invasive IP catheters (4) inserted into the spinal cord.



4.1.c Spinal cord injury procedure

An articulating arm (660, Starrett, Athol, Massachusetts, USA) was fixed to the T9, T10, and T11 positioned pedicle screws with titanium rods. This arm positions in place the impact device, which consists of an impactor (diameter, 0.953 cm) fitted with a load cell (LLB215; Futek Advanced Sensor Technology, Irvine, CA) that slides down a guide rail equipped with a Balluff Micropulse® linear position sensor (BTL6-G500-M0102-PF-S115, Balluff Canada Inc., Mississauga ON, Canada) to record the force and impactor position from which displacement and velocity were determined. Immediately after the weight-drop contusion injury (weight 50 g; height 50 cm), sustained compression was maintained on the contused spinal cord for 30 min by placing an additional 100g mass onto the impactor (150 g total) (Figure 4).

Figure 4. A schematic figure of the experimental setup that shows position of the impactor.



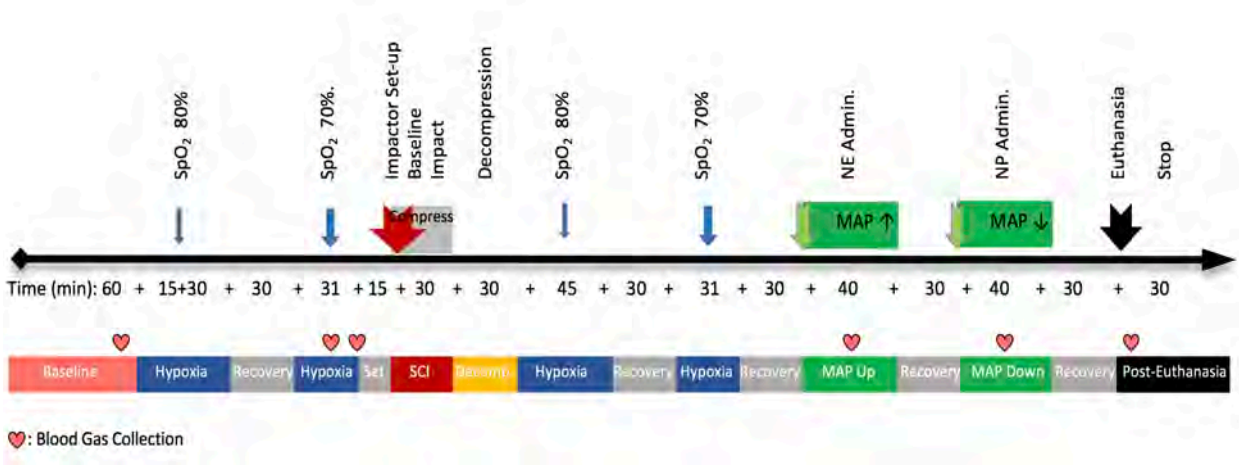
4.1.d Experimental Protocol

Once the placement of intraparenchymal probes was finalized, we allowed a 60-minute stabilization period before beginning a 90-minute baseline-recording period prior to SCI. NIRS measures of spinal cord tissue oxygenation (O_2Hb, HHb, Hbdiff, TOI%) and hemodynamics (THb) were monitored during serial episodes of hypoxia and MAP

alterations before and after inducing an acute SCI at T10. Simultaneous "gold standard" IP measures of oxygenation (partial pressure of oxygenation; $PaPO_2$), perfusion (spinal cord blood flow; SCBF), and hydrostatic pressure were also monitored to compare against the NIRS parameters.[1] Lactate and pyruvate levels were measured with an Iscusflex Microdialysis Analyzer (CMA, Stockholm, Sweden), using the manufacturer's reagents and standard protocols (Figure 5).

First, mild hypoxia was induced by adjusting the N_2:O_2 until the animal's SaO_2 dropped to 80%, after which the pig was returned to normal ventilated respiration until all levels were stable. Next, severe hypoxia was induced by turning off the oxygen and all other ventilation assistance (pig not breathing) until animal's SaO_2 dropped to 70%, followed by a return to normal ventilated respiration with oxygen until all levels were stable again. As described previously in this experimental model for generating acute SCI, the maximum impact force applied to the exposed spinal cord at the tip of the impactor on average is around 5500 kdynes.[1] Such contusion-compression injury results in a compete loss of gray and white matter at center of the impact (T10), with loss of white and gray matter at distances up to 10–13mm from the epicenter in both rostral and caudal directions. MAP was increased and decreased by 20 mmHg for 30 min periods, by intravenous injection of NE and NP respectively. A 30-minute recovery time was allowed after each episode. Arterial and venous blood were collected for blood gas analysis at baseline, the peak of the pre-SCI severe hypoxia and after 30 minutes of recovery time following hypoxia.

Figure 5. Experimental protocol, including: two episodes of mild (SaO_2 80%) and severe (SaO_2 70%) hypoxia, SCI and sustained cord compression, cord decompression, post-SCI mild (SaO_2 %80) and severe (SaO_2 %70) hypoxia episodes followed by two episodes of MAP increase and decrease.



General timeline of Experiment 1 were as follow:

- 06:00am Animal placement and anesthesia
- 07:00am Urinary catheter placement
- 07:45am Femoral catheters placement
- 09:00am Jugular & carotid catheters placement
- 10:00am Laminectomy surgery
- 12:30pm Spinal cord ultrasound
- 12:45pm IP and NIRS sensors placement
- 02:00pm Data stabilization start
- 03:00pm Anesthesia stabilization
- 03:30pm Baseline measure
- 05:00pm Protocol starts
- 11:00pm Euthanasia
- 11:30pm Experiment end

4.1.e Data analysis and statistics.

All data, including NIRS measures of spinal cord chromophore concentrations (O_2Hb, HHb), spinal cord IP measures ($PaPO_2$, SCBF, SCP) and vital signs (SaO_2, heart rate, respiratory rate) were recorded continuously during the experimental interventions and recovery periods. The raw optical data were converted into changes in NIRS parameters (O_2Hb, HHb, CCO), and THb, Hbdiff and TOI% were calculated via the NIRS software.

Arterial and venous blood gas analysis was used for calculation of capillary oxygen saturation percentage ($S_{cap}O_2$) levels, based on a reference ratio between the arterial (25%) and venous contribution (75%) to the signal.[2] $S_{cap}O_2$ measures at baseline, acute hypoxia peak and recovery time were compared with changes of $PaPO_2$ as well as NIRS-derived oxygenation parameters (O_2Hb, HHb, Hbdiff and TOI%) during same episodes.

The changes for each variable during each event, including spinal cord NIRS-derived O_2Hb, HHb, CCO, THb, Hbdiff, TOI%, and spinal cord IP $PaPO_2$, SCBF, SCP and L/P ratio were compared using Wilcoxon signed-rank test to determine statistical significance. Pearson correlation coefficients were calculated to establish the pairwise relationships between the IP and NIRS measurements during episodes of induced hypoxia, and MAP changes. Combined-intervals sensitivity and specificity of NIRS parameters for predicating positive and negative changes of $PaPO_2$ and SCBF were performed. Data are presented as means ± standard error (SEM) and the level of significance set at $p<0.05$ for all statistical analysis and comparisons. Data were analyzed using SAS v9.4 (SAS Institute, Cary, North Carolina).

4.2. Results

Nine anesthetized adult female Yorkshire pigs with mean weight of 27.9±1.34 kg were studied. NIRS derived measures of spinal cord oxygenation and hemodynamics (O_2Hb, HHb, CCO, Hbdiff, TOI% and THb), measured by the customized miniature multi wavelength OXT5 sensor, were successfully monitored during experimental interventions in all nine animals. NIRS sensor placement and on-going monitoring were not associated with any complications.

4.2.a Injury parameters

The average peak force applied to the exposed spinal cord was 8253.98 ± 701.26 kdynes with an impulse of 17.78 ± 0.25 kdyne*sec. The impactor tip traveled 7.76 ± 0.23 mm from initial contact with the exposed dura with a velocity of 2970.76 ± 22.94 mm/sec at impact. The individual biomechanical impact parameters of the animals are presented in Table 3.

Table 3. Measures of weight and injury parameters. SEM, standard error of the mean.

Animals #	Body weight (kg)	Impulse (kdynes*s)	Impact velocity (mm/s)	Displacement (mm)	Max force (kdynes)
1	31.0	17.22	2958.76	8.58	4990.79
2	25.0	18.89	3025.99	6.20	10027.64
3	26.0	16.34	3017.17	7.83	8724.26
4	36.0	17.37	2962.98	8.48	6598.37
5	28.0	18.03	3004.94	7.66	10492.53
6	23.0	18.09	3027.20	7.85	9244.92
7	29.5	17.81	2975.10	7.91	10322.33
8	24.0	17.84	2803.40	7.51	7556.48
9	29.0	18.40	2961.29	7.82	5428.52
Mean	**27.9**	**17.78**	**2970.76**	**7.76**	**8153.98**
SEM	**1.34**	**0.25**	**22.94**	**0.23**	**701.26**

4.2.b IP Parameters.

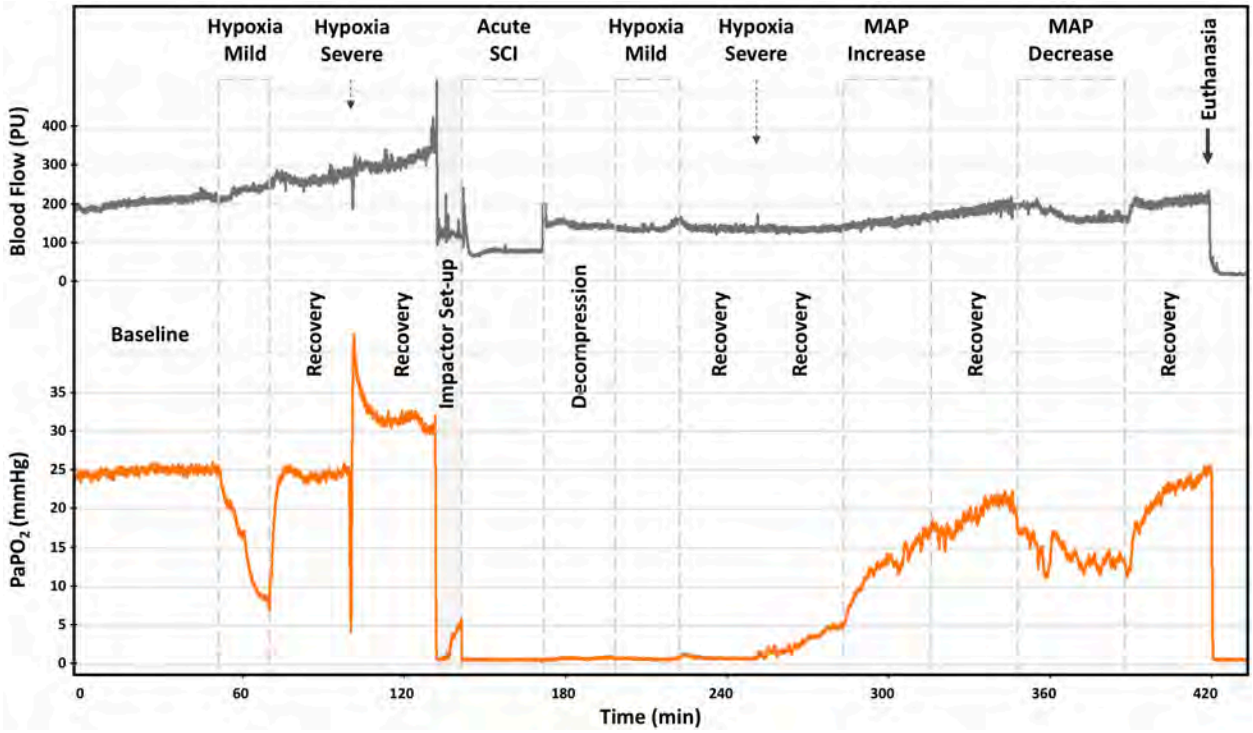
Changes in spinal cord $PaPO_2$ were statistically significant during all episodes and changes of SCBF were significant during both episodes where MAP was increased or

decreased. SCP were significantly changed during cord decompression and where MAP was decreased. (Table 4). A representative example of changes of $PaPO_2$ and SCBF during sequential experimental interventions is shown in Figure 6.

Table 4. Changes of spinal cord $PaPO_2$, SCBF, SCP and L/P ratio during episodes of the experiment. All values are mean±SEM; * shows measures with significant changes ($p<0.05$).

	$\Delta PaPO_2$	ΔSCBF	ΔSCP	ΔL/G
Hypoxia 1	-17.93 ± 2.35*	21.88 ± 13.05	0.15 ± 0.15	-0.96 ± 0.42
Hypoxia 2	-19.85 ± 3.18*	0.17 ± 11.81	0.49 ± 0.38	-0.39 ± 0.84
SC Decompression	14.44 ± 5.01*	62.06 ± 49.86	-8.36 ± 3.19*	-79.69 ± 3.25*
Hypoxia 3	-13.50 ± 4.43*	23.33 ± 21.08	-0.04 ± 0.54	-10.20 ± 1.07
Hypoxia 4	-16.45 ± 13.66*	-9.47 ± 11.28	0.10 ± 0.32	-1.97 ± 0.79
MAP Increase	9.53 ± 3.33*	49.61 ± 14.51*	1.20 ± 1.06	-3.22 ± 0.97*
MAP Decrease	-14.74 ± 5.02*	-58.92 ± 20.19*	1.64 ± 0.41*	6.05 ± 2.11*

Figure 6. Changes of IP-derived SCBF and $PaPO_2$ during the entire experimental protocol in animal #3. In this animal, $PaPO_2$ dropped to zero upon the impact and failed to recover for close to 4 hours (~ 130 – 250 min).



4.2.c NIRS parameters.

Mean changes of NIRS-derived O_2Hb, HHb, Hbdiff and TOI% are shown in Figures 7 and 8. during interventions of the protocol. The Pearson correlation analysis, combined-intervals correlation between $S_{cap}O_2$ and NIRS-derived spinal cord O_2Hb, HHb, Hbdiff and TOI% showed that, correlations between $S_{cap}O_2$ and O_2Hb, HHb, Hbdiff, TOI% were "R=0.89, n=9, p=0.0000", "R=0.85, n=9, p=0.0000", "R=0.88, n=9, p=0.0000", and "R=0.77, n=9, p=0.0007" respectively (Figure 9). The correlation between $S_{cap}O_2$ and $PaPO_2$ was also analyzed, (R= 0.88, n=9, p=0.0000).

Figure 7. Mean changes of NIRS-derived O_2Hb and HHb during interventions of the protocol. * P<0.05

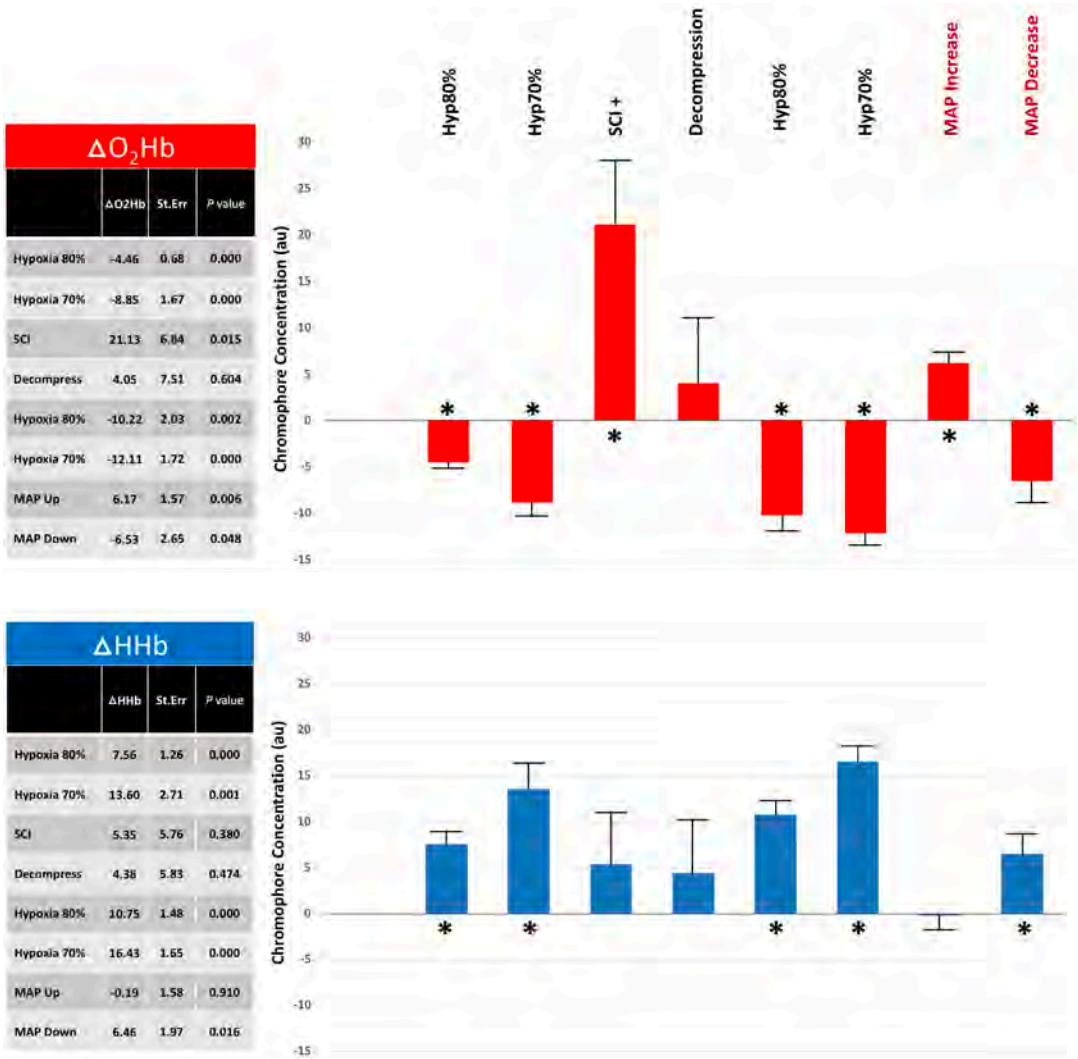
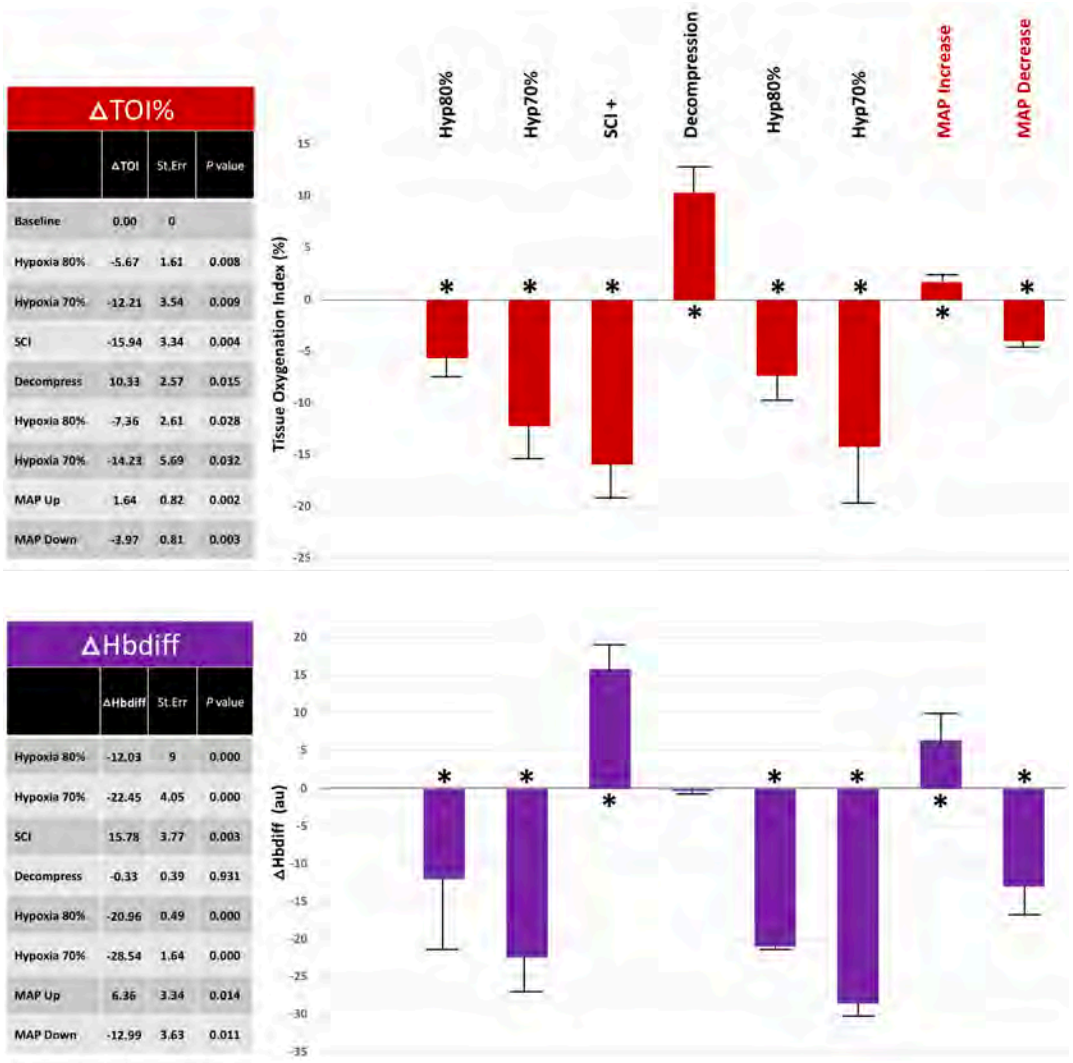


Figure 8. Mean changes of Hbdiff and TOI% during interventions of the protocol. * P<0.05



Changes in NIRS-derived O_2Hb, HHb, Hbdiff and TOI% during each experimental intervention are shown in Table 5. Changes in TOI% were statistically significant during all events. Changes in spinal cord O_2Hb and Hbdiff were statistically significant during all episodes of hypoxia and alterations in MAP. A representative figure of NIRS parameter changes during the experiment is shown in Figure 10. Changes in THb were significant during pre-SCI hypoxia and increases in MAP.

Figure 9. combined-intervals correlation between $S_{cap}O_2$ and NIRS-derived spinal cord O_2Hb, HHb, Hbdiff and TOI%.

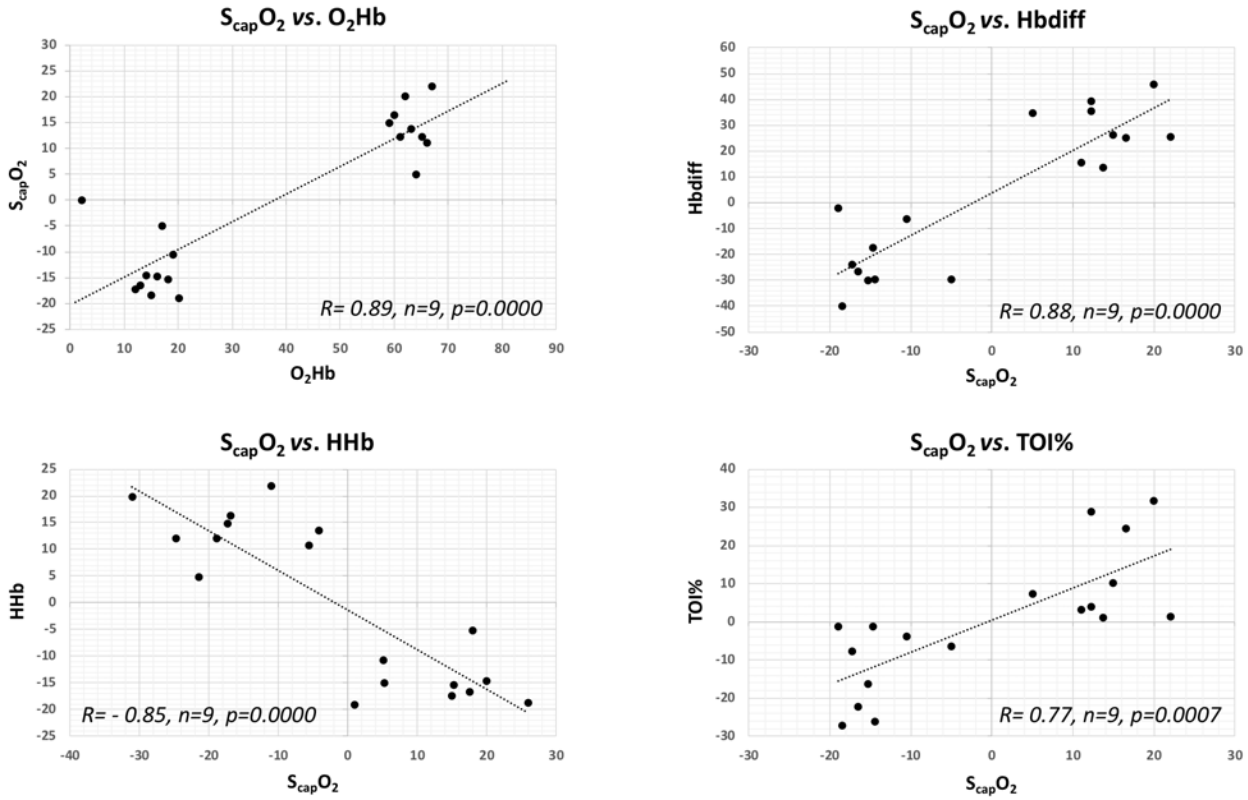
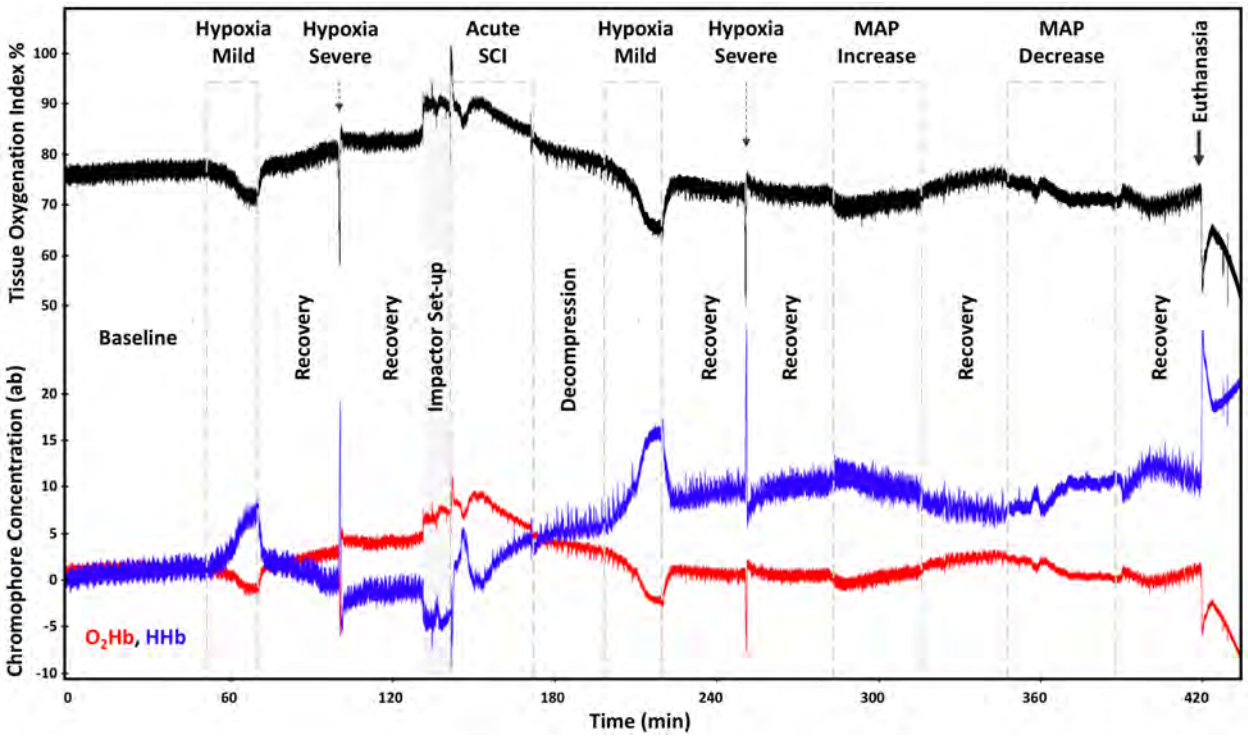


Table 5. Changes of spinal cord O_2Hb, HHb, Hbdiff and TOI% during episodes of the experiment. All values are mean±SEM; * shows measures with significant changes ($p<0.05$).

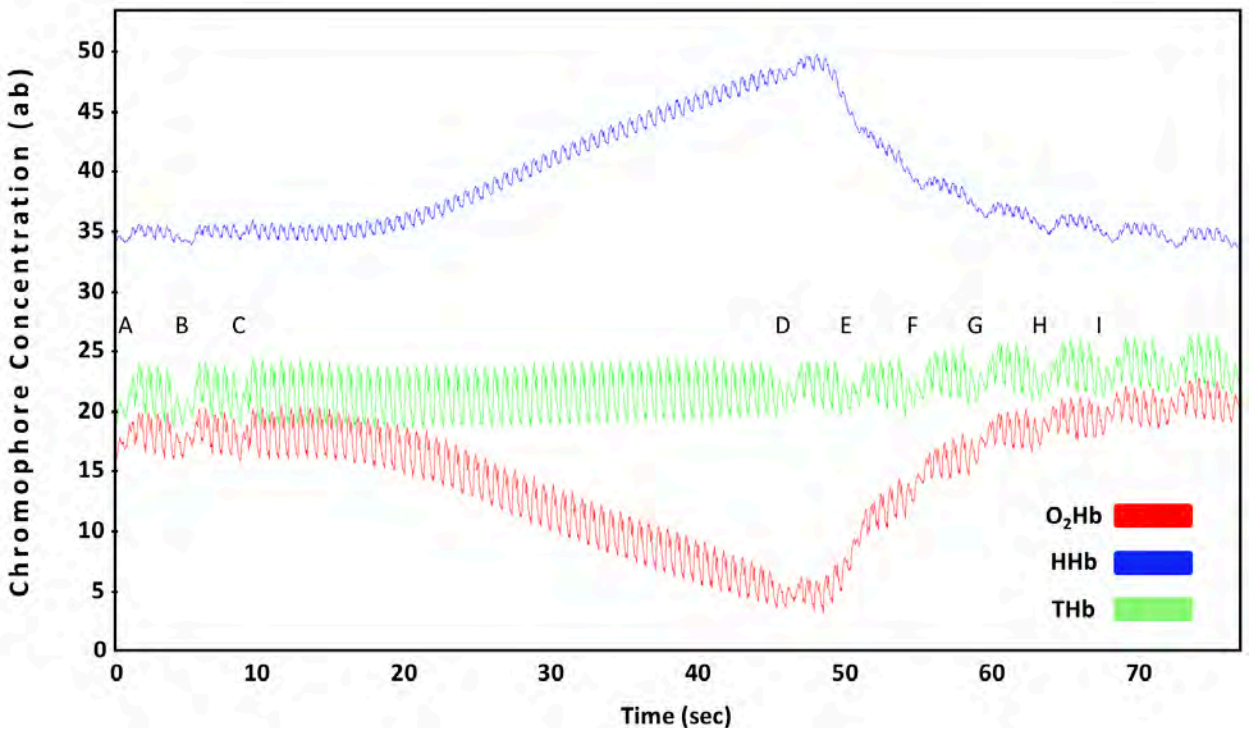
	ΔO₂Hb	ΔHHb	ΔHbdiff	ΔTOI%	ΔTHb	ΔCCO
Hypoxia 1	-4.46 ± 0.68*	7.56 ± 1.26*	-12.03 ± 9*	-5.67 ± 1.61*	3.10 ± 1.31*	-0.11 ± 0.13
Hypoxia 2	-8.85 ± 1.67*	13.60 ± 2.71*	-22.45 ± 4.05*	-12.21 ± 3.54*	4.74 ± 1.99*	-0.21 ± 0.10
SC Decompression	4.05 ± 7.51	4.38 ± 5.83	-0.33 ± 0.39	10.33 ± 2.57*	8.41 ± 12.93	-0.56 ± 1.33
Hypoxia 3	-10.22 ± 2.03*	10.75 ± 1.48*	-20.96 ± 0.49*	-7.36 ± 2.61*	5.35 ± 2.33	-0.05 ± 0.28
Hypoxia 4	-12.11 ± 1.72*	16.43 ± 1.65*	-28.54 ± 1.64*	-14.23 ± 5.69*	4.34 ± 2.46	-0.25 ± 0.12
MAP Increase	6.17 ± 1.57*	-0.19 ± 1.58	6.36 ± 3.34*	1.64 ± 0.82*	5.99 ± 2.47*	0.53 ± 0.21*
MAP Decrease	-6.53 ± 2.65*	6.46 ± 1.97*	-12.99 ± 3.63*	-3.97 ± 0.81*	0.09 ± 3.05	-0.61 ± 0.21*

Figure 10. Changes of NIRS-derived O_2Hb, HHb and TOI% during the entire experimental protocol in animal #3.



Chromophore changes occurring when severe hypoxia was induced (SaO_2 reduced to 70%) are shown in Figure 11. This figure illustrates the sensitivity of the MW-NIRS prototype in detecting physiological changes within the spinal cord tissue before, during and after the hypoxic event.

Figure 11. Changes in spinal cord O_2Hb and HHb, and THb during an episode of severe hypoxia (C-D). The high-resolution data, collected at 100 Hz, capture cardiac pulsations within the spinal cord tissue (i.e. each fine oscillation), and the effect of ventilation on spinal cord tissue oxygenation (i.e. respiratory cycles of A-B, B-C, D-E,…).



4.2.d. IP vs. NIRS parameters.

NIRS-derived changes in HHb, O_2Hb, Hbdiff, and TOI% in the spinal cord following induction of hypoxia, and after re-oxygenation, correspond to the changes in spinal cord $PaPO_2$ measured via the IP catheter during both periods of hypoxia. Combined-intervals correlation between invasive IP measure of spinal cord $PaPO_2$ and noninvasive NIRS measures of spinal cord O_2Hb, HHb, Hbdiff and TOI% are shown in Figure 12. Combined-intervals sensitivity and specificity of NIRS parameters for predicating positive and negative changes of $PaPO_2$ and SCBF are included in Table 6.

Figure 12. Pearson Correlation analysis between PaO_2 and NIRS-derived O_2Hb, HHb, Hbdiff and TOI%.

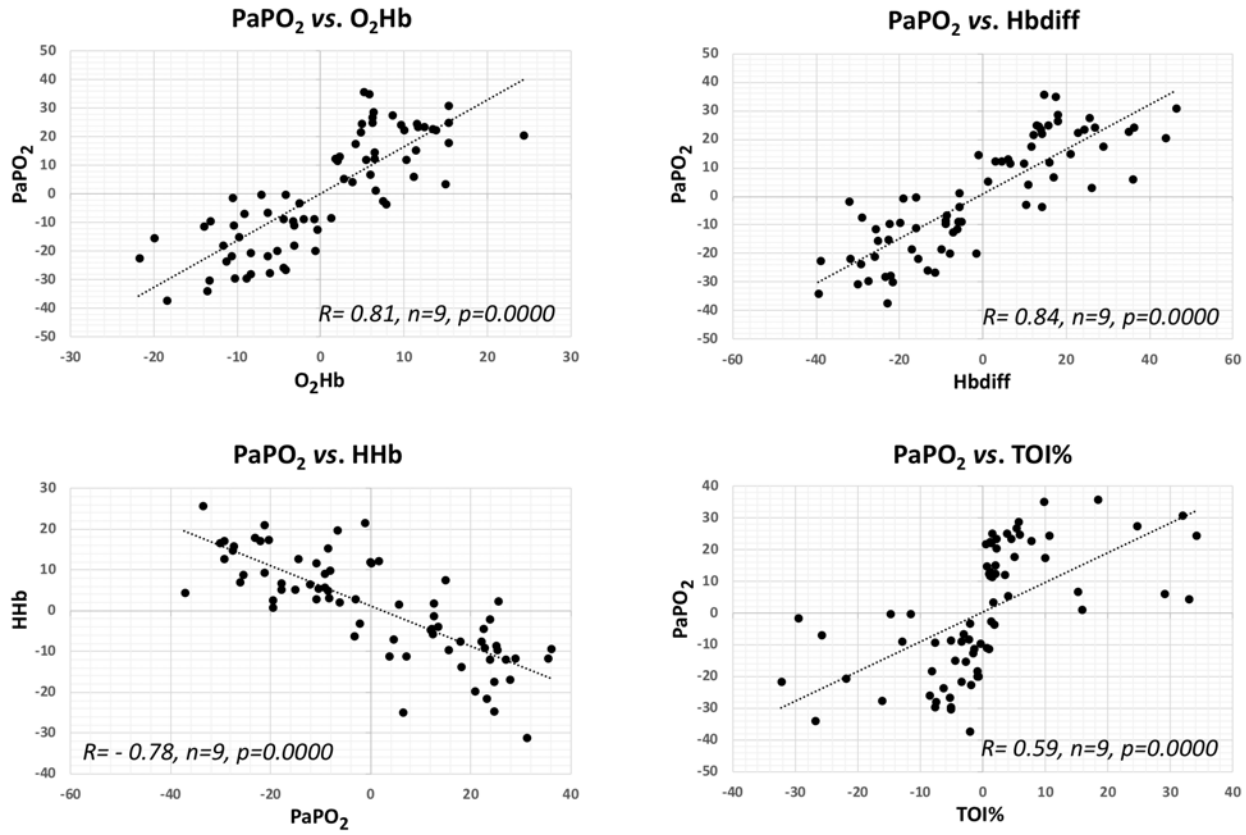


Table 6. Combined-intervals sensitivity, specificity, positive predicting value (PPV) and negative predicting value (NPV) for NIRS parameters predicating IP measures of $PaPO_2$ and SCBF.

NIRS Measures Predicting Positive IP Measures

	Sensitivity	Specificity	PPV	NPV
$\Delta PaPO_2$ *vs.* ΔO_2Hb	100.00	92.10	91.89	100.00
$\Delta PaPO_2$ *vs.* ΔHHb	14.70	5.26	12.20	6.45
$\Delta PaPO_2$ *vs.* ΔHbdiff	94.12	94.74	94.12	94.74
$\Delta PaPO_2$ *vs.* ΔTOI%	100.00	89.47	89.47	100.00
ΔSCBF *vs.* ΔTHb	73.17	50.00	61.22	63.33

NIRS Measures Predicting Negative IP Measures

	Sensitivity	Specificity	PPV	NPV
$\Delta PaPO_2$ *vs.* ΔO_2Hb	92.10	100.00	100.00	91.89
$\Delta PaPO_2$ *vs.* ΔHHb	5.26	14.70	33.33	35.55
$\Delta PaPO_2$ *vs.* ΔHbdiff	94.74	94.12	94.74	94.12
$\Delta PaPO_2$ *vs.* TOI%	89.47	100.00	100.00	89.47
ΔSCBF *vs.* ΔTHb	50.00	73.17	63.33	61.22

EXPERIMENT 2

Technology Development Round 3.

A miniaturized version of OXT5 NIRS sensor (V2-NIRS sensor) is designed and developed by our NIRS technology developer contractor (Figure 13). This new sensor includes a smaller (diameter: 3.2 mm) multi wavelength light source (MW-LED) that is customized for our project by an optics company in China. This sensor also benefit a smaller sensitive photodetector. Two small N52 magnets are mounted at the head of this sensor. These magnets couple to magnetic arm of a fixator (Figure 14) developed by our team for placement and stabilization of V2 sensor over the spinal cord during a week of continuous monitoring. The sensor is connected to the NIRS hardware through a 2.5m shielded multi-branch customized cable. This set up is being tested in a series of pilot animal acute and survival studies.

Figure 13. V2-NIRS sensor prototype.

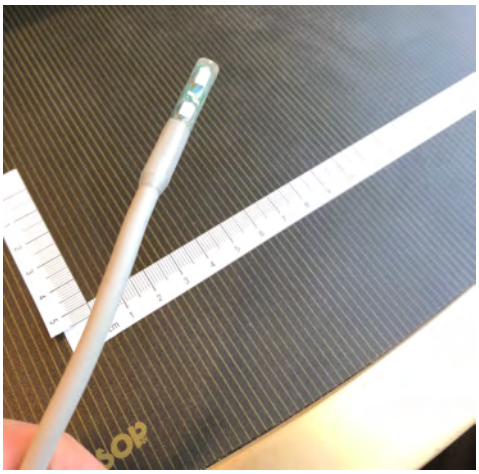
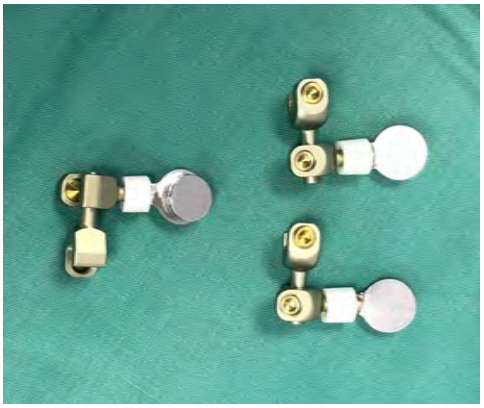


Figure 14. Magnetic V2-NIRS sensor fixator system.



5 KEY RESEARCH ACCOMPLISHEMENTS

- OXT5 NIRS sensor and system was successfully developed and tested.
- Research protocol of Experiment 1 was tested and refined during a pilot study.
- Experiment 1 is completed as scheduled.
- OXT5 NIRS sensor could collect a novel trace of Cytochrome C activity with the spinal cord.
- OXT5 was able to successfully collect validated pattern of tissue hypoxia, from the spinal cord, immediately after induction of ventilatory hypoxia.
- Analysing the data a consistent relationships between NIRS and IP measures are observed.
- V2 NIRS sensor and system that would be applied in Experiment 2 are developed and are being tested in a series of pilot animal studies.
- A novel magnetic fixator for placement and fixation of V2NIRS sensor over the dura for Experiment 2 is designed and successfully developed.

6 CONCLUSIONS

This research focuses on establishing a novel NIRS-based system and sensor that can be used to monitor spinal cord hemodynamics in the early post-injury period. Such an intervention will enable clinicians to optimize patient care by providing them real-time information about the physiology of the injured spinal cord. We have been successful to achieve our second year goals; Experiment 1 was successfully completed. The outcomes of Experiment 1 suggest that the proposed transdural MW-NIRS technique has the potential to monitor spinal cord oxygenation and hemodynamics noninvasively. Upon successful completion and data analysis of Experiment 1 and testing new miniaturized V2-NIRS sensor, we will conduct Experimment 2 in Year 3.

7 PUBLICATIONS, ABSTRACTS, PRESENTATIONS

Podium Presentations:

Presented:

1. *"Optical monitoring of spinal cord subcellular damage after acute spinal cord injury" Babak Shadgan, Neda Manouchehri, Kitty So, Katelyn Shortt, Femke Streijger, Andrew Macnab, Brian Kwon.*
   Optical Diagnostics and Sensing Conference, SPIE Photonics West Congress, San Francisco, USA, Feb 2018.

2. "Attenuation of near infrared light by fibrin bioadhesive", Macnab A.J., Panago R., Kwon B., Dumont G., Shadgan B.
   Optical Diagnostics and Sensing Conference, SPIE Photonics West Congress, San Francisco, USA, Feb 2018.

Accepted for Podium Presentation:

1. *"Studying the effects of mean arterial pressure changes on spinal cord hemodynamics in a large animal model of acute spinal cord injury, using a novel optical sensor." Presentation#: 568.22.*
   Neuroscinece 2018, San Diego, November 6th, 2018

2. *"Changes of mean arterial pressure affect spinal cord oxygenation as monitored by an implantable near-infrared spectroscopy sensor in an animal model of acute spinal cord injury."*
   Optical Diagnostics and Sensing Conference, SPIE Photonics West Congress, San Francisco, USA, February 2nd, 2019.

Publications:

Published:

1. Shadgan B, Manouchehri N, So K, Shortt K, Streijger F, Macnab A, Kwon B. *Optical Monitoring of Spinal Cord Subcellular Damage After Acute Spinal Cord Injury.* Proc. SPIE, 105010L (Feb. 2018); doi: 10.1117/12.2286551.
   https://doi.org/10.1117/12.2286551

2. Macnab A.J., Panago R., Kwon B., Dumont G., Shadgan B. *Attenuation of near infrared light by fibrin bioadhesive.* Proc. SPIE, 105010M (Feb. 2018); doi: 10.1117/12.2286563.
   https://doi.org/10.1117/12.2286563

In progress:

- *"Optical assessment of spinal cord tissue oxygenation using a miniaturized near infrared spectroscopy sensor".* Babak Shadgan Andrew Macnab, Allan Fong, Neda Manouchehri, Kitty So, Katelyn Shortt, Femke Streijger, Peter A. Cripton, Eric C. Sayre, Guy A. Dumont, Roberto Pagano, Kyoung-Tae Kim, Brian K. Kwon. Prepared for submission to J Neurotrauma, on October 2018.

8 INVENTIONS, PATENTS AND LICENSES

To explore the potential intellectual property that might be resulted from this project we approached the UBC University-Industry Liaison Office (UILO) and have filed an Invention Disclosure & Assignment application (file number of 17-037) in the Q1 period. Our invention disclosure was assessed by the UILO. The assessment included prior art searchers using the Thomson Innovation database as well as PubMed and Google Scholar for relevant publications. The prior art search identified a number of NIRS-based methods for monitoring spinal cord. However, none of them were similar to our design and capability of integrated real-time monitoring of spinal cord hemodynamics, oxygenation, and Intraparenchymal pressure. The UILO has determined that our system design is patentable. We are planning to revisit our potential IPs upon completion of Experiment. We are also planning to apply for an IP related to our MW-NIRS calculation method in 2019.

9 REPORTABLE OUTCOMES

Nothing to report

10 OTHER ACHIEVEMENTS

Nothing to report

11 REFERENCES

1. Streijger, F., So, K., Manouchehri, N., Tigchelaar, S., Lee, J.H.T., Okon, E.B., Shortt, K., Kim, S.E., McInnes, K., Cripton, P., Kwon, B.K. (2017). Changes in Pressure, Hemodynamics, and Metabolism within the Spinal Cord during the First 7 Days after Injury Using a Porcine Model. J Neurotrauma 34, 3336-3350.

2. Pollard, V., Prough, D.S., DeMelo, A.E., Deyo, D.J., Uchida, T., Stoddart, H.F. (1996). Validation in volunteers of a near-infrared spectroscope for monitoring brain oxygenation in vivo. Anesth. Analg. 82, 269-277.

12 APPENDICES

Appendix 1 – Proposal for Design and Development of a Custom-made NIRS System (OXT5).

Pathonix Innovation Inc.
033-2366 Main Mall
Vancouver, BC, V6T 1Z4 Canada
Phone: (604) 210-7279 Ext 101

Proposal for the Design and Revision of a Custom-Made NIRS System with Electrical Probe for Long Term Monitoring of the Spinal Cord in an Animal Model of SCI

Prepared for Dr. Brian Kwon
February 8, 2018

SUMMARY

This proposal is for the redesign of the NIRS probe and system used in Spinal Cord NIRS Study-Experiment 1 to accommodate long term recording in live animal cases. This proposal assumes an electrical probe will be used for experiment 2. Currently, use of an optical probe is being evaluated separately and independently. The detailed proposals for each technique will be provided for Client's consideration.

ABOUT PATHONIX

Pathonix Innovation Inc. is a Canadian company specializing in Near Infrared Spectroscopy (NIRS) and Pulse Oximetry technology and its applications in biomedical, health research and sports monitoring. Our innovative solutions have received a number of national and international awards and our wireless tissue oxygen monitoring systems are currently in use in several universities and research facilities in North America. Pathonix benefits from physics, engineering, medical and exercise science expertise and is led by a team with track record of research, technology and product development. Through partnership with other well-established engineering companies and consultants, Pathonix has access to a wide range of resources for product design, prototyping and manufacturing. The company also has a successful history of collaboration with university researchers and is currently working on novel applications of NIRS and pulse oximetry in health care and exercise monitoring.
Pathonix experience in development of commercial medical pulse oximeters from concept to contract manufacturing, experience in collaborative research with well-known research facilities and universities and past experience in IP development in the field of NIRS and pulse oximetry makes it a good fit for executing the project outlined in this proposal.

REQUIREMENTS

The spinal cord NIRS study – experiment 2 requires long term (up to a week) continuous data collection on live and mobile animals. This imposes specific requirements on the probe and the data collection device. Additionally, experiment 1 exposed some weaknesses and limitations that need to be addressed for the next phase. These requirements are outlined below.

- Probe refinement
  The main limitations of the probe have been identified as size, reliability, robustness and ease of scaling as needed. Therefore, the following objectives will be met for the next round.
    - 20 probes for the target 10 cases (1 per case plus one spare probe for each case)

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- Waterproofing, modifying the probe tip, adding biocompatible epoxy for better water/fluid ingress protection and improved cable strain relief. It should be noted that there is a trade-off between size and probe robustness. We will provide quantitative measures of the strain the probe can withstand, as well as water resistance limits as part of the pilot studies. If higher strain or ingress protection is needed, the size may need to increase.
- Robust probe-to-unit connection to avoid accidental data loss as a result of animal movements.
- Minimizing the probe form factor
  - replacing the LED with a smaller package. We have identified a supplier that can produce custom made 5 wavelength LEDs in a package smaller than the ones used in the first phase (3.5 mm X 2.8 X 1.8mm compared to 5.5 mm X 5 mm X 1.6). The minimum order quantity (MOQ) for these custom LEDs is 1000 units, with a negotiated total price of 2k USD.
  - Replacing the double cable with a custom cable with 8 inner conductors and shield and more flexible jacket material. We have also identified a potential supplier for such a cable. The MOQ for this cable is 500m with the negotiated price of 2USD/meter. The cable change will result in better ingress protection in the probe compared to 2 separate cables.

- Software/firmware changes
  - Raw data processing performed on the device and only downsampled data saved to reduce file sizes and disk usage for long-term continuous data collection
  - Optional wireless and battery powered capability addition for better mobility and lower likelihood of damage to the cable or probe due to motion, and onboard data saving to prevent data collection interruption in case of cable disconnection.
  - Light weight, faster and easier to use user interface.
  - Improved high speed and robust data connection
  - Alarm feature for selected parameters
  - Automatic real time data saving with time stamp

- Hardware changes
  - Automatic gain control addition: The variability observed in tissue properties during experiment 1 may result in variations in the quality of the data collected. An automatic control mechanism will be implemented in the next revision to adjust for variations in tissue properties between cases.
  - Improved analog frontend performance: The noise immunity of the analog front end will be improved to prevent interferences from other equipment's operating around the subject.
  - Probe board revision to reduce size and accommodate new and smaller LED: As described above, the design of the smaller probe requires changes in the probe PCB design.

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BUDGET

The project expenses are outlined below.

- Revising probe tip design, prototyping costs and testing, manufacturing 20 probes: 8k
- Revising analog boards, probe boards and prototyping expenses: 7k
- Software/Firmware changes and updates: 10k
- Custom-made cable cost, shipping and duties: 1.5k USD
- Custom-made LED cost, shipping and duties: 2.5k USD

Optional additions are outlined below.

- Wireless, battery powered and onboard data saving capability addition: 8k
- Extra probes: 250 CAD/probe
- Project will be delivered with 4 NIRS units. Extra NIRS unit will be charged at 2500 CAD/unit as required.

Similar to experiment 1, Pathonix will conduct the project under a professional services contract. This phase of the project will be billed at a non-recurring total price of $25,000 CAD plus $4,000 USD for the LEDs and cable. The optional portion of the project can be completed at an additional total value of $8,000 CAD upon approval. This development will be performed mostly at Pathonix facilities in Vancouver, BC. The testing of the device and animal data collection will be collected at the facilities provided by the Client. Pathonix will use its own computers, technical tools, equipment and software for the design and development of the device apart from any dedicated tool (operating computers, reference devices, patient simulators etc.) provided by the Client.

Pathonix bills the Client at 3 major milestones. The breakdown of the invoices is as follows: $5,000 CAD at the project kickoff to cover the upfront components expenses and shipping costs, $10,000 CAD upon delivery of the first probe iteration to be used in the pilots (milestone 1), $7,000 CAD upon delivery of the revised probes and devices for the experiments (milestone 2), and $3,000 CAD upon completion of the experiments and delivery of the final probes, devices and report (milestone 3 and project closure). Please note that GST will be charged at 5% as required by Canada Revenue Agency.

The team members who will be working on the project are listed in the table below (subject to change as needed).

Team Member	Expertise	Experience	Role
Behnam Molavi	Electrical/ Biomedical Engineering (PhD)	Biomedical signal processing, Medical Device development, Biophotonics and in particular Pulse oximetry and NIRS	Project management, NIRS data analysis, overall system design and testing, MATLAB UI design, help with data collection and internal verifications
Kevin Reilly	Chemical/Mechanical Engineering (PhD)	Product development, Mechanical design and prototyping and supply chain management	probe design and manufacturing, Mechanical prototyping, component sourcing, Phantom development
Shayesteh Vefagh	Biomedical Engineering (BSc.)	Embedded systems, analog systems, Electrical design	Firmware developer, PCB design and manufacturing, electrical prototyping and testing

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Deliverables

The project will be concluded upon delivery of the following:

Software

Custom made user interface optimized for long term data collection. The software will be provided as a standalone executable as well as a package containing the source code. The code will be sufficiently commented to allow further development/modifications by the Client.

Sensor Hardware

20 prototype sensors and 4 NIRS units will be delivered. Each device measures ΔO_2Hb, ΔHHb, ΔTHb, %TOI and ΔCCO in real time and can record data for as long as 1 week.

System Specifications and final report

Detailed optical and electrical specifications will be provided for future use in the study. This includes details such as minimum required LEDs intensity, minimum photodetector specifications, amplifier specifications (signal to noise ratio, gain etc.) and other required technical details.

FINANCIAL HEALTH

Date Range: From 1-SEP-2017 to 31-AUG-2018			
	Actual Expenditures	Commitments	Projected Actuals
	YTD	YTD	
Funding/Revenues			
Carry Forward Allocation	349,920.05	0.00	349,920.05
Expense Funding Allocation	814,407.69	0.00	814,407.69
Total Funding/Revenues	1,164,327.74	0.00	1,164,327.74
Expenses			
Salaries			
Faculty Salaries	24,421.02	0.00	24,421.02
Student Salaries	9,345.00	0.00	9,345.00
Staff Salaries	110,852.38	0.00	110,852.38
Sessional Salaries	100,127.55	0.00	100,127.55
Total Salaries	244,745.95	0.00	244,745.95
Benefits	43,018.51	0.00	43,018.51
Supplies & Sundries	217,581.74	13,908.15	231,489.89
Capital Expenditures			
Equipment	25,709.67	23,290.45	49,000.12
Computing Eq & Software	3,203.05	0.00	3,203.05
Minor Renovations	1,740.75	0.00	1,740.75
Total Capital Expenditures	30,653.47	23,290.45	53,943.92
Travel	5,061.21	0.00	5,061.21
Professional Fees	3,230.00	0.00	3,230.00
Total Expenses	544,290.88	37,198.60	581,489.48
Revenue Less Expenses	620,036.86	-37,198.60	582,838.26